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CLOSURE OF CRANIAL ARTICULATIONS IN THE SKULL OF THE AUSTRALIAN ABORIGINE

By A. A. ABBIE, *Department of Anatomy, University of Adelaide*

INTRODUCTION

While it is well known that joint closure advances more or less progressively with age, there is still little certainty in matters of detail, mainly for lack of adequate series of documented skulls. In consequence, sundry beliefs have arisen which tend to confuse the issue. One view, now disposed of (see Martin, 1928), is that early suture closure indicates a lower or more primitive type of brain. A corollary, due to Broca (see Topinard, 1890), that the more the brain is exercised the more is suture closure postponed, is equally untenable. A very widespread belief is based on Gratiolet's statement (see Topinard, 1890; Frédéric, 1906; Martin, 1928; Fenner, 1939; and others) that in 'lower' skulls the sutures are simple and commence to fuse from in front, while in 'higher' skulls the sutures are more complicated and tend to fuse from behind. This view was disproved by Ribbe (quoted from Frédéric, 1906), who substituted the generalization that in dolicocephals synostosis begins in the coronal suture, and in brachycephals in the lambdoid suture. In addition to its purely anthropological interest the subject raises important biological considerations of brain-skull relationship, different foetalization in different ethnological groups (see Bolk, 1926; Weidenreich, 1941; Abbie, 1947), and so on.

A survey of the literature reveals very little in the way of data on the age incidence of suture closure. The only substantial contribution accessible here comes from Todd & Lyon (1924) for Europeans, but their work is marred by arbitrary rejection of awkward material. Ribbe's thesis (quoted by Todd & Lyon) is not available in Adelaide. There are practically no data for the Australian aborigine. The most extensive observations are by Fenner (1939), but are only incidental and without age check. The series reviewed here is smaller than Fenner's but has the advantage of including an appreciable number of juveniles and adolescents, while, for the adults, Campbell's (1939) correlation of dental attrition with age in living aborigines has been employed.

MATERIAL AND METHODS

The material comprised 206 skulls, fifty-four from the Department of Anatomy, University of Adelaide, and 152 from the South Australian Museum. These represent about 20% of all the skulls available. They were taken as they came, strictly at random, and were subsequently found to include thirty-seven juveniles and adolescents, seventy-seven females and probable females, and ninety-two males and probable males. Campbell's (1925) criteria rendered allocation of the juveniles and adolescents into 5-year groups fairly simple. Age classification of the adults on dental attrition was much less precise because of overlapping of the grades. Broca's grades of attrition—I, II, III and IV—were employed by Campbell (1939), who found the age distribution in 300 living aborigines as given in Table 1.

Table 1

Age group (years)	I	II	III	IV
12–20	20	16	—	—
20–29	33	33	9	—
30–49	12	34	46	14
50 and over	1	15	38	28

As might be expected, there is a progressive shift from grade I to grade IV with advancing age, but no certainty in any individual case. There are two further difficulties. In the first place the degree of attrition, particularly in young adults, was often far from uniform, mainly because of the later eruption of the back teeth. Also, Campbell found that as a rule the females showed a greater degree of attrition than males of corresponding age. His figures are as shown in Table 2. Such complications obviously interfere with the precision of age estimates, and those for adults can be no more than approximate. However, they are the sole criteria of age available and are considerably better than nothing at all.

The distribution of the skulls employed is shown in Table 3.

Table 2

Age group		I	II	III	IV
20–29:	Males (53)	31	19	3	—
	Females (19)	3	11	5	—
30–49:	Males (67)	11	31	22	3
	Females (44)	2	8	23	11
50 and over:	Males (57)	—	13	25	19
	Females (18)	1	1	8	8
Totals:	Males (177)	42	63	50	22
	Females (81)	6	20	36	19

Table 3

Estimated age*	0–5	6–10	11–15	16–20	20±		20–29		30–49		50+	
Attrition†	—	—	—	—	I		II		III		IV	
Sex	—	—	—	—	F.	M.	F.	M.	F.	M.	F.	M.
No. of skulls	11‡	10	5	11	15	6	23	37	31	35	8	14
Totals	J. 37				F. 77				M. 92			

* For males; females probably a little younger.

† Dental attrition was only employed as a criterion in skulls with complete adult dentition.

‡ Including one abnormal skull of dental age 4–5 years; it was very elongated and narrow, with protuberant frontal and occipital regions.

Todd & Lyon (1924), following the lead of Parsons & Box (1905), insisted upon the necessity for taking the time of closure from the endocranial rather than the ectocranial condition. Martin (1928) states that only human skulls commence closure at the inner table before the outer. He points out, however, that generally speaking endocranial closure of individual sutures runs parallel with ectocranial closure, but that there may be a few regional differences. Of these skulls, the museum specimens could not be opened, and it was necessary to be content with the ectocranial pattern. Such skulls as could be examined from the inside did not show such a constant

advance in endocranial closure as Parsons & Box describe. For the sake of consistency only ectocranial closure is considered here.

For each suture five possible conditions could be recognized: grade 1, completely open; grade 2, commencing closure; grade 3, more advanced closure; grade 4, complete closure; grade 5, complete closure and obliteration. These grades are relatively arbitrary, but they provided a useful means of comparing one suture with another, or parts of the same suture.

Todd & Lyon (1924) classified the cranial sutures into three different functional groups: (*a*) sutures of the vault—metopic, coronal, sagittal and lambdoid; (*b*) circummeatal sutures—spheno-temporal (inferior part), squamous, parieto-mastoid and masto-occipital; (*c*) accessory—spheno-parietal and spheno-frontal.

Careful consideration of the skulls under examination, and comparison with the times of closure given by Todd & Lyon, suggest the following scheme as more logical.

In the first place, the vault of the skull is separated from the base by what is—particularly in the Australian aborigine—a virtually horizontal suture complex (Fig. 1A) comprising the fronto-nasals, the zygomatico-frontals, the spheno-frontals and spheno-temporals (where this sequence exists), the squamo-parietals, masto-parietals and the lambdoid. This complex may well be called 'the great horizontal suture'. As opposed to this are the sutures of the vault—metopic, coronal and sagittal on the one hand (Fig. 1B); and the joints of the base—spheno-occipital, temporo-sphenoid and masto-occipital on the other (Fig. 1C). Of the three basal joints the spheno-occipital is wholly between cartilage bones, the masto-occipital mainly so, and the temporo-sphenoid partly so. The articulations of the vault and base close appreciably earlier than the members of the great horizontal suture, both in the Todd & Lyon series and in this. Exceptions are found where the systems meet at the pterion and lambda. In addition, the great horizontal suture is notable for the presence of a number of fontanelles, and it is the major site for Wormian bones which are, incidentally, especially common at the pterion, asterion and lambda.

OBSERVATIONS

The great horizontal suture

Naso-frontal. In only three cases, all female, was there any fusion in this suture. Fusion was incomplete and there was no apparent age relationship.

Zygomatico-frontal. Complete fusion of this suture was never observed, but the posterior part may be involved in the general spread from the pterion.

Spheno-frontal and spheno-parietal. The pteric part of the sphenoid is considered as a whole, since it seems to behave the same whether the pattern is spheno-parietal, squamo-frontal or includes an epipteric complex. Closure here is regional rather than sutural and generally involves the pars pterica of the coronal suture as well, as shown in Table 4.

Despite individual fluctuations a definite pattern emerges from these figures. The onset of closure here before the age of about 15 years is apparently always regional. Thereafter the association of sphenoidal with pteric fusion, while still marked, falls off in both sexes. With advancing age, however, the female gradually recovers the juvenile association while the male diverges from it in a progressive manner.

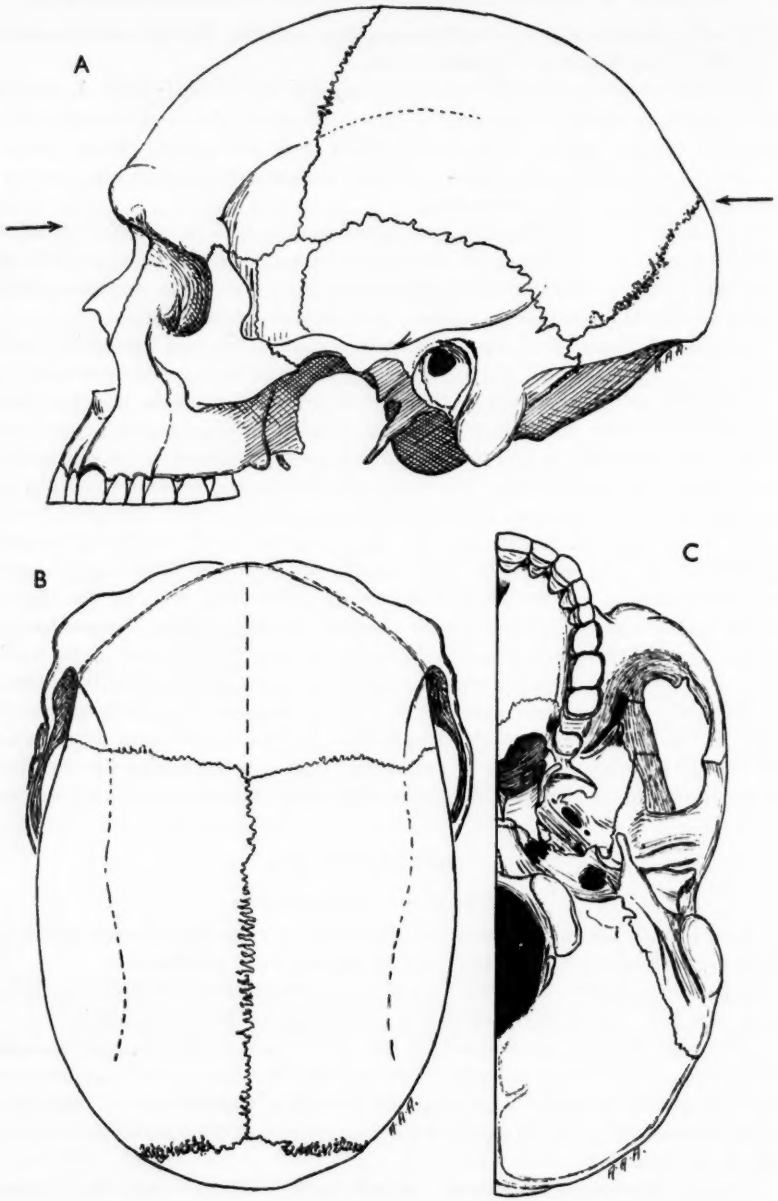

Fig. 1. The skull of an Australian aborigine (from Loxton, South Australia), drawn from three aspects to illustrate the classification employed in this paper ($\times \frac{1}{2}$ approx.). The arrows in A indicate the great horizontal suture. *Note:* (*a*) that the styloid and mastoid processes are unusually well developed for an Australian aborigine; (*b*) that pars pterica of the coronal suture was approaching obliteration but has been emphasized here in the interests of clarity.

Evidently two patterns of closure are in progress: an early one related to the pterion and a later reflecting the general ageing of the skull.

Squamo-parietal. Only ten out of the 206 skulls showed any trace of fusion, and they included the abnormal infant already mentioned. The nine adults with some closure betrayed no apparent correlation with age. Five of the adults were involved to the degree of grades 2–3 in the anterior half or third of the suture—clearly a spread from pteric fusion. Conversely, in the abnormal infant, while the posterior two-thirds of this suture was completely closed (grade 5), the anterior one-third conformed to the pteric condition of grade 3 closure. In the remaining four adults the squamo-parietal suture showed grade 2 fusion throughout—in harmony with the generally advanced condition of these skulls as a whole.

Table 4

Age	0–5	6–10	11–15	16–20	—		—		—		—	
Attrition	—	—	—	—	I		II		III		IV	
Sex	—	—	—	—	F.	M.	F.	M.	F.	M.	F.	M.
Percentage of skulls showing some closure	20*	60	40	80	87	83	96	84	75	89	100	93
Percentage correlation with pars pterica of coronal	100	100	80	80	80	83	90	81	75	60	100	50

* Including the abnormal skull.

Table 5

Age	0–5	6–10	11–15	16–20	—		—		—		—	
Attrition	—	—	—	—	I		II		III		IV	
Sex	—	—	—	—	F.	M.	F.	M.	F.	M.	F.	M.
Percentage with some closure in pars lambdica	10	10	—	20	33	17	44	81	83	89	73	86
Percentage with some closure in pars media	—	—	—	20	25	17	33	76	72	77	73	79
Percentage with some closure in pars asterica*	—	—	—	—	7	17	9	32	45	54	43	36
Percentage with complete closure*	—	—	—	—	7	—	4	—	7	23	30	—
Percentage correlation at lambda	90	80	90	91	91	100	80	100	83	82	62	57

* Excluding closure of the asterion itself; this was completely closed in only six skulls.

Masto-parietal. This portion of the bounding articulation of the mastoid extends from the squamo-mastoid junction to the asterion and is really a joint between cartilage bone and membrane bone. Under 20 years of age only two skulls (one abnormal) showed any closure. Of the adults, four females and sixteen males showed fusion, but without any definite age correlation. Closure was usually slight only (grade 1), and in ten skulls (including the abnormal infant) was clearly associated with the condition of the adjoining squamo-parietal suture. Only one grade IV male showed complete obliteration of the whole mastoid suture, and this in contrast to a fully squamo-parietal and almost equally open adjoining lambdoid suture.

Lambdoid. This is usually divided into three parts—lambdica, media and asterica—and the percentage of closure in the different parts is shown in Table 5.

The trend of closure from the pars lambdica towards the pars asterica is fairly clear in both sexes, although it is comparatively rare for the asterion itself to become wholly obliterated. The regional influence is seen in the high association with closure of the pars lambdica of the sagittal suture. This is particularly so in childhood and early adulthood, but falls in late adulthood in both sexes. Here, again, there appear to be two kinds of closure: a primary, correlated with spread from the lambda—more characteristic of juveniles, and a secondary—dissociated from the lambda and more evident in advanced age. The female seems to attain complete closure earlier than the male.

The sutures of the vault

Metopic. In one infant of less than 1 year the anterior fontanelle was large; the posterior half of the metopic suture was fully open, the anterior half practically obliterated. In an infant of less than 18 months' dental age the fontanelle was much smaller; the posterior half of the metopic suture was obliterated, the anterior half partially so (grade 3). Beyond that age there was no trace of a fontanelle and the condition of the suture varied. All the infants under 5 years, except the abnormal, showed some trace of the nasal end (ranging from $\frac{1}{2}$ in. to a mere suspicion), and for all practical purposes the suture may be considered closed by an age of under 2 years. Thereafter, no complete metopic suture was found in any of the juveniles, and only eighteen of the adults showed even a trace (varying from $\frac{1}{2}$ to $\frac{1}{8}$ in.) at the nasal end.

Coronal. This suture is divided into thirds—pars bregmatica, pars complicata and pars pterica. Most commonly the upper two-thirds—bregmatica and complicata—occupy the interval between bregma and the temporal line, while the pars pterica extends from the line to the pterion. In a number of cases, however, the temporal line cuts the suture near the middle, more or less bisecting the pars complicata. The stephanion, then, is not a fixed point in the Australian aborigine, but there are no present data on any correlation with such factors as skull height, etc. The condition of the coronal suture depended to some extent upon its relation to the temporal line. Thus, from bregma to stephanion—whether two-thirds or a half—tended to behave as one unit, while from stephanion to pterion—whether a third or a half—tended to behave as another. Table 6 gives the percentage of closure of its various parts.

Table 6

Age	0–5	6–10	11–15	16–20	—		—		—		—	
Attrition	—	—	—	—	I		II		III		V	
Sex	—	—	—	—	F.	M.	F.	M.	F.	M.	F.	M.
Percentage with some closure in pars pterica	20*	60	40	82	100	100	96	97	100	100	100	100
Percentage with some closure in pars complicata	10	60	—	55	40	100	61	68	86	86	88	100
Percentage with some closure in pars bregmatica	10	10	—	28	40	33	48	68	71	83	88	93
Percentage with complete closure	—	—	—	—	20	—	26	11	28	43	38	7
Percentage correlation at bregma	90	80	100	82	87	83	87	77	76	77	75	86

* Including the abnormal skull.

Evidently, closure may commence very early in the pars pterica of the coronal suture and in the young skulls shows a very high correlation with closure of the pterion as a whole (Table 4). This association persists to a slightly less extent in adults but remains more uniformly evident in females than in males. Progress in adolescence is accompanied by extension of closure into the pars complicata, and in late adolescence and adulthood into the pars bregmatica. This early closure pattern was the commonest, prevailing in 66 % of all adults of both sexes up to the age of 50 years. In 5 % of adults, however, closure starts at the bregma simultaneously with, or slightly after, the pterion. Such bregmatic closure was twice as common in males as in females and had a high correlation with the condition of the bregma generally. In 1 % closure starts at the bregma alone, and in 1 % at the pars complicata. Over the age of 50 the coronal suture in females was completely obliterated except for a trace at the temporal line (grade 4) in 50 %. In males of the same age 20 % showed complete closure, 50 % were closing from bregma and pterion simultaneously and the remaining 30 % pursued the sequence from below up. Here, again, two patterns are detectable. Before the age of 20 years 80 % of skulls showing coronal fusion begin to fuse at the pterion. After that age this juvenile pattern becomes overlaid by an increasing incidence of bregmatic closure. The female appears to be ahead of the male in coronal fusion as a whole and retains a preference for the pterion, while the male tends progressively more towards the bregma.

Sagittal. This suture is usually divided into approximate fourths: pars bregmatica, pars verticis, pars obelica and pars lambdica. Fusion may start in any one or two of the sections excluding, apparently, the pars verticis. The percentages at the various sites are given in Table 7.

Table 7

Age	0–5	6–10	11–15	16–20	—		—		—		—	
Attrition	—	—	—	—	I		II		III		IV	
Sex	—	—	—	—	F.	M.	F.	M.	F.	M.	F.	M.
Percentage with some closure in pars bregmatica	20*	10	—	64	40	17	48	73	80	89	75	93
Percentage with some closure in pars verticis	27*	20	20	55	40	50	48	78	83	82	75	93
Percentage with some closure in pars obelica	27*	27	40	27	40	50	70	90	90	94	88	100
Percentage with some closure in pars lambdica	20*	27	20	27	40	17	70	84	90	91	88	93
Percentage with complete closure	10*	—	—	—	—	—	9	14	24	37	30	50
Percentage correlation at bregma	90	80	100	82	87	93	87	77	76	77	75	86
Percentage correlation at lambda	90	80	90	91	91	100	80	100	83	82	62	57

* Including abnormal skull.

These figures indicate that early synostosis of the sagittal suture usually begins anteriorly and spreads back. In adults this pattern persists in a high proportion, but more in males than in females. Correlation with the condition of the bregma generally was very high in juveniles and young adults. There is little positive sexual

distinction, but in advanced age the males seem ahead of the females. A secondary pattern of posterior closure may appear—either *ab initio* or imposed upon the primary pattern. Posterior closure begins slightly more commonly in the pars obelica than in the pars lambdica, and was usually more evident in males than in females. Correlation with the lambda shows a male preponderance in young adults but little sexual difference in later years. The sagittal suture may, then, begin fusion as early as the coronal, but the incidence before adulthood is much lower—33 % as compared with 80 %. In adults, the male was nearly always ahead of the female, and appeared to show a greater preference for posterior closure, particularly in the pars obelica.

The articulations of the base

Here the position is rather changed by a high incidence of joints between cartilage bones.

Spheno-occipital. This, being between purely cartilage bones, is a joint *sui generis*, and has little necessary relation to the condition of the other articulations. In the abnormal skull of less than 5 years synostosis was already complete. Otherwise no evidence of closure could be found until 11–15 years, when some 40 % showed commencing fusion. Before the end of adolescence more than half the skulls betrayed some closure and in a third it was complete. In females over 20 years the joint remained open a little longer—in one case up to an age of about 25 years. In males there was no trace of an open spheno-occipital joint over the age of 20.

Spheno-temporal. The inferior part of this joint is in cartilage, the remainder in membrane bone. Observations were made on only fifty skulls comprising two juveniles, one adolescent, and nineteen adult females and twenty-eight adult males of all grades of dental attrition. The joint is conveniently divided into thirds: the upper two-thirds from pterion to infra-temporal crest and a lower third from crest to petrous temporal. In this series some closure was found in only 33 % of all cases. Almost always fusion began in the lower third, spreading up towards the pterion; an exception was a single grade II female with closure in the upper third only—clearly a spread from pteric fusion. The single adolescent showed grade 4 fusion in the infra-temporal third only. In adult females closure incidence of any part of the joint did not exceed 25 % at any age. The adult males, however, gave a 20 % incidence at grade II, 60 % at grade III but 25 % at grade IV (eight skulls only). In one grade IV female closure extended to the upper two-thirds of the joint, but this was found in eleven grade III and IV males. In one grade IV male the whole suture was obliterated up to the pterion, and in this case both the pteric end of the sphenoidal and the lower third of the coronal suture were also obliterated, so that, apparently, pteric fusion had been superimposed upon the usual pattern.

Masto-occipital. This represents approximately the lower two-thirds of the whole mastoid articulation and is almost entirely in cartilage bones. Broadly speaking, one-half lies on the postero-lateral aspect of the skull—below the asterion, while the lower half is inferior—medial to the mastoid process. Closure of this suture was always from the antero-inferior end, up and back towards the asterion. Up to 10 years about 10 % showed some closure in the lower half and by 20 years 40 % gave evidence of fusion there. In adults there is a sexual difference. Both sexes at

grade I attrition showed a 50 % incidence of closure; thereafter, the female incidence did not rise above 66 % at any age, while the male incidence increased progressively to over 90 % after the age of 50. The only case of complete closure—from end to end —was in a male of grade IV attrition.

Regional closure

Since the foregoing figures indicate that joint closure is much more a regional than a sutural process it is of interest to compare the age and sex incidence of fusion in the regions most obviously concerned, as may be done in Table 8.

Table 8

Age	0–5	6–10	11–15	16–20	—		—		—		—	
Attrition	—	—	—	—	I		II		III		IV	
Sex	—	—	—	—	F.	M.	F.	M.	F.	M.	F.	M.
Percentage with some closure at pterion	20*	60	40	80	87	83	96	84	75	89	100	93
Percentage correlation†	100	100	80	80	80	83	90	81	75	60	100	50
Percentage with some closure at bregma	20*	10	—	64	40	17	48	73	80	89	75	93
Percentage correlation	90	80	100	82	87	83	87	77	76	77	75	86
Percentage with some closure at lambda	20*	27	20	27	40	17	70	84	90	91	88	93
Percentage correlation	90	80	90	91	91	100	80	100	83	82	62	57

* Including abnormal skull.
† Percentage corresponding in condition with the adjoining sutures.

These figures indicate that the predominant pattern is initial closure at the pterion to a progressive degree with age but to a greater extent in females. In both sexes the incidence of initial closure at the bregma and lambda is below that at the pterion. There is very little obvious preference as between bregma and lambda at any age for the site of closure, but in most cases the female lags behind the male, which latter shows an increasing preference of the sagittal suture over the coronal with advancing age. The late fall in correlation at the lambda and pterion appears to indicate a tendency to replace regional by sutural closure in later life.

Additional observations

There was nearly always some asymmetry in suture closure on the two sides of the skull. In the vast majority the left side was ahead of the right by one or two grades; only rarely was the right ahead of the left. Three skulls showed an especially marked disparity in the coronal suture. In two grade II females with the left suture still moderately open (grades 2–3) the right was wholly obliterated (grade 5); in one grade II male the right was moderately open but the left was obliterated.

It is commonly assumed that the skull of the Australian aborigine, being 'primitive', has simple sutures. This, however, is only a partial truth, for many of these skulls showed a quite complex condition of portions of the coronal, sagittal and lambdoid sutures.

DISCUSSION

Classification of the cranial articulations into those of the vault separated from those of the base by a 'great horizontal suture' finds some justification in the observations recorded here. The great horizontal suture has some special characters. In the first place, it joins a region wholly of membrane bone to one which contains a large element of cartilage bone. Then, it is a major site for fontanelles and Wormian bones. And finally, the elements of the horizontal suture complex as a whole show synostosis much later than the joints of either the vault or the base; exceptions occur at two points of contact—the pterion and lambda. The joints of the vault and base may begin fusion well before the end of adolescence. But, while fusion of those of the vault usually starts at their junction with the great horizontal suture, those of the base generally begin fusion inferiorly, in their more purely cartilaginous portion, and gradually extend upwards towards that suture. Consequently, the pterion and lambda show relatively early closure, but the asterion remains open until late in life and, indeed, is rarely found wholly obliterated.

In 80–90 % of skulls under the age of 50 years suture closure is regional rather than sutural. The heaviest incidence of closure falls upon the pterion, and beyond the age of about 20 years few skulls were found without some closure there. The bregma and lambda lag behind the pterion into late adolescence, but in adult life their incidence of closure, while still lower than at the pterion, shows a progressive and rapid increase to about 90 % in later life. In this respect the male appears to outstrip the female. Over 50 years of age, closure tends to become more independently sutural.

Fenner examined the ectocranial condition of 672 male and 564 female aboriginal skulls. He found the sutures simple. The metopic suture was present in about 0·7 % of females and 0·6 % of males. There was complete fusion of coronal, sagittal and lambdoid sutures in seventeen females and twenty-one males, and of the coronal, sagittal and pars lambdica of the lambdoid in twenty-two females and forty-six males. Most advanced closure occurred in the sagittal suture in 45 % of females but 73 % of males, and in the coronal in 52 % of females but 26 % of males. There was complete synostosis of lambdoid and coronal sutures in 1 %, while the lambdoid was more advanced than the coronal and sagittal in only 2 % of females but in 14 % of males. The temporal part of the coronal suture was more advanced in 83 % of cases, the bregma in 7 %, the temporal and bregma together in 8 % and the pars complicata in 1 %. Closure of the sagittal suture started in the pars obelica and spread thence anteriorly and posteriorly, while the pars bregmatica was usually the last to fuse—here synostosis was most advanced in 18 % of females and 7 % of males.

Fenner's findings are in close agreement with those recorded here. Two points of importance arise. First, the female tends to cling to the juvenile pattern of anterior closure while the male shows an increasing preference for posterior closure. This female tendency towards the juvenile pattern of closure may be considered as further evidence in favour of the view that the female skull generally preserves foetal characters longer than the male (Abbie, 1947). The other point is that the female skull, which has a higher mean cephalic index than the male skull (see Abbie,

1947), has a more anterior pattern of closure than the male. This is a contradiction of Ribbe's thesis that longer skulls close anteriorly first, shorter skulls posteriorly. Further, the abnormal skull—if it may be cited as evidence—is more decidedly contrary to Ribbe's views. The thesis may, indeed, be restated thus: that more juvenile skulls begin closure antero-inferiorly, more mature skulls postero-superiorly.

In 1236 skulls Fenner found an overall incidence for the metopic suture of 0·7 % as compared with Anutchin's figure of 1 % (see Martin, 1928). The present series of 206 skulls showed not a single instance of complete metopic suture, even in the infant under 1 year of age. Moreover, the writer does not recall a single complete metopic suture in over 1000 aboriginal skulls examined in the past few years. It appears, then, that the figure given for the aborigine is too high. Both the metopic suture and the spheno-occipital joint appear to close a little earlier in the Australian aborigine than in the European.

The observations of Todd & Lyon (1924) do not include any on adolescents or juveniles, and these writers have rejected material they considered unsuitable. Also, they preferred endocranial closure to ectocranial closure as a criterion. Subject to these provisos they discovered that in Europeans closure starts in the pars obelica of the sagittal suture at 22 years and is practically complete at 31. The coronal begins to close at 24 and is usually complete at 38—the pars pterica being last to disappear. The lambdoid suture starts at 26 and is complete at 42, but not at the asterion until 47. In the case of the coronal and sagittal sutures the picture in aborigines who begin closure early—particularly females—is the reverse of that in Europeans; but where the onset of closure is late—particularly in males—the aboriginal condition more nearly approaches the European. Further, the European lambdoid suture—part of the great horizontal suture—is practically the same as the aboriginal, while, at the pterion, the spheno-parietal and spheno-temporal begin to close early in Europeans—at 22 and 29 years respectively—as they do in aborigines. Of the sutures of the base the aboriginal spheno-occipital is slightly ahead of the European, but the masto-occipital is about the same in both groups.

It is interesting to note that the main difference between the two patterns concerns the vault much more than the base or the great horizontal suture. This last, indeed, can scarcely be distinguished in the two groups. Altogether, even allowing for the fact that the criteria for comparison are not quite the same, it is not unreasonable to assume that in many aboriginal skulls joint closure is not so decidedly different from that in Europeans as is usually supposed.

SUMMARY

1. An account is given of the approximate time of ectocranial joint closure in 206 Australian aboriginal skulls comprising thirty-seven juveniles and adolescents, seventy-seven females and ninety-two males.

2. Age estimates were based upon dental criteria. Order of eruption was employed for juveniles and adolescents. All skulls with complete permanent dentition were treated as adults and classified according to Broca's grades of attrition in correlation with Campbell's observations upon living aborigines.

3. The cranial articulations were grouped as:

(*a*) those of the vault—metopic, coronal and sagittal; separated from

(*b*) those of the base—spheno-occipital, spheno-temporal and masto-occipital; by

(*c*) a great horizontal suture complex comprising the naso-frontals, zygomatico-frontals, spheno-frontals, spheno-parietals, squamo-parietals, masto-parietals and lambdoid suture.

4. The elements of the great horizontal suture as a whole close later than those of the vault and base, but they behave the same at the points of contact—pterion, asterion and lambda—in 80–90 % of skulls.

5. In some 80–90 % of skulls under 50 years of age suture closure is regional rather than sutural. The main order of incidence is pterion, bregma and lambda, and asterion last. The juvenile pattern is closure of the pterion with spread up to the bregma and then posteriorly. Adult females tend to preserve this order, but adult males show an increasing preference for the obelion-lambda, thereby moving towards the European pattern. There is much overlapping of patterns. Over the age of 50 years both sexes betray a tendency towards independent sutural closure, especially at the pterion and lambda. Complete closure of the asterion is rare.

6. Both the spheno-occipital and the metopic joints close a little earlier than in Europeans. The incidence of persistent metopic suture is probably much less than the figure of 1 % usually quoted.

7. The generalization concerning anterior and posterior suture closure seems to be best expressed thus: the younger the skull at the time of onset of closure the more closure tends to begin anteriorly.

I wish to express my thanks to the Director of the South Australian Museum for providing access to his skeletal material and to Miss Pamela Harley and Miss Jean Chappel for assistance in recording these observations.

REFERENCES

Abbie, A. A. (1947). Headform and human evolution. *J. Anat., Lond.*, **81**, 233–258.

Bolk, L. (1926). *Das Problem der Menschwerdung.* Jena: Fischer.

Campbell, T. D. (1925). Dentition and palate of the Australian aboriginal. *Univ. Adel. Publ. No.* 1 *under Keith Sheridan Foundation.*

Campbell, T. D. (1939). Food, food values and food habits of the Australian aborigines in relation to their dental conditions. Part IV. Jaws and dentition. *Aust. J. Dent.* **43**, 141–156.

Fenner, F. J. (1939). The Australian aboriginal skull: its non-metrical morphological characters. *Trans. roy. Soc. S. Aust.* **63**, 248–306.

Frédéric, J. (1906). Untersuchungen über die normale Obliteration der Schädelnähte. *Z. Morph. Anthrop.* **9**, 373–454.

Martin, R. (1928). *Lehrbuch der Anthropologie*, 2te Aufl., Bd. **2**. Jena: Fischer.

Parsons, F. G. & Box, C. R. (1905). The relation of the sutures to age. *J. R. anthrop. Inst.* **35**, 30–38.

Todd, T. Wingate & Lyon, D. W. (1924). Endocranial suture closure. *Amer. J. phys. Anthrop.* **7**, 325–384.

Topinard, P. (1890). *Anthropology.* London: Chapman and Hall.

Weidenreich, F. (1941). The brain and its role in the phylogenetic transformation of the human skull. *Trans. Amer. phil. Soc.*, N.S., **31**, 321–442.

THE FLEXOR MUSCLES OF THE FOREARM AND HAND IN LIZARDS AND MAMMALS

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INTRODUCTION

The phylogeny of the muscles of the forearm and hand has been reviewed recently in a general way by Howell (1936*a*), and that of the flexors of the forearm in some detail by Straus (1942), and these authors give full references to the earlier literature. But many questions remain unanswered, largely on account of the lack of precise myological and neurological information. Among lizards it is only in the case of *Iguana* that detailed drawings of all the muscles of the hand are available (in the fine monograph of Rabl, 1915). In mammals the muscles are better understood, but even here many details are unknown, and the precise courses of the smaller nerve trunks have never been figured. The purpose of this paper is to provide detailed illustrations of the arrangement of the muscles and nerves in a lizard and a mammal, and to make suggestions as to the morphological changes that have occurred in the course of mammalian phylogeny.

The lizard *Varanus* and the mammal *Felis* have been chosen for special study as they are large, readily available animals often mentioned in the literature. *Varanus* is a particularly suitable type for comparison with mammals, as one of the phalanges of digit III and two of digit IV have been reduced, bringing the toes more into line (see Fig. 4), and the muscular insertions have been simplified by the loss of tendons originally attached to these phalanges. Similar dwarfing of phalanges is well known in several mammal-like reptiles, and must have occurred in the line leading to mammals, as the phalangeal formula was reduced from the typical reptilian 2:3:4:5:3 to the 2:3:3:3:3.

Detailed references to the literature will be found in the text. In addition, Dr W. L. Straus, Jr. has been so kind as to allow full use of his unpublished notes on the hands of *Sceloporus* and *Ctenosaura*, work which, taken together with his 1942 paper, gives a complete picture of the muscles of these forms, allowing a detailed check on the work presented here.

OBSERVATIONS

Pronator teres and flexor carpi radialis

In *Varanus* (Fig. 1) the pronator teres ('flexor antibrachii radialis' of Straus, 1942) passes from the humerus to the lower two-thirds of the radius, and the flexor carpi radialis to the radiale. In other lizards and in *Sphenodon* the muscles are similarly arranged, but Straus found the flexor carpi radialis reaching the first metacarpal in *Sceloporus*. The two muscles are separated by the emergence of the marginal radial branch of the nervus flexorius ('n. brachialis longus inferior' of Straus), which also supplies them. In *Felis* (Fig. 5), as in most mammals, the muscles pass to the middle

of the shaft of the radius and to metacarpals II and III, and there is no nerve between them. All authors are agreed that these muscles are homologous in reptiles and mammals.

Epitrochleo-anconeus and flexor carpi ulnaris

In *Varanus* and other lizards these muscles pass from the humerus to the upper part of the ulna and to the pisiform bone. In *Felis*, and in mammals in general, an additional head of the flexor carpi ulnaris arises from the upper end of the ulna, but otherwise the muscles resemble those of lizards, and there is no doubt of their homology. They are always supplied by the ulnar nerve.

Palmaris longus

In *Varanus* the palmaris longus is a large, well-defined muscle. It arises from the medial epicondyle of the humerus and is inserted on the superficial band of the flexor retinaculum of the wrist. It is supplied by a special branch of the median nerve. The muscle may be absent in lizards, *Ctenosaura* and *Sceloporus* (Straus, 1942), or present as an individual variation, as in *Iguana*, where Howell (1936*a*, p. 293) found it in one individual, and Rabl (1915, Taf. 19D) has figured it as a split-off part of the flexor carpi ulnaris which joined the palmar aponeurosis. On the other hand, Ribbing (1907) has figured a well-developed muscle in *Tejus*, and described it in *Tupinambis* and *Ameiva* as well as in *Varanus*. Further, he found a corresponding muscle, his 'Flexor primordialis, Teil A', with its characteristic nerve supply from the median, in *Crocodilus*, an entirely unrelated reptilian type.

Straus (1942, p. 291) considered the palmaris longus a new mammalian muscle, usually derived from the flexor digitorum sublimis. But in view of its frequent occurrence in reptiles, where a sublimis has never been evolved, it seems more probable that the palmaris longus was a part of the muscular equipment of the primitive reptilian-mammalian stem, and that it has been inherited by the surviving groups that still possess it.

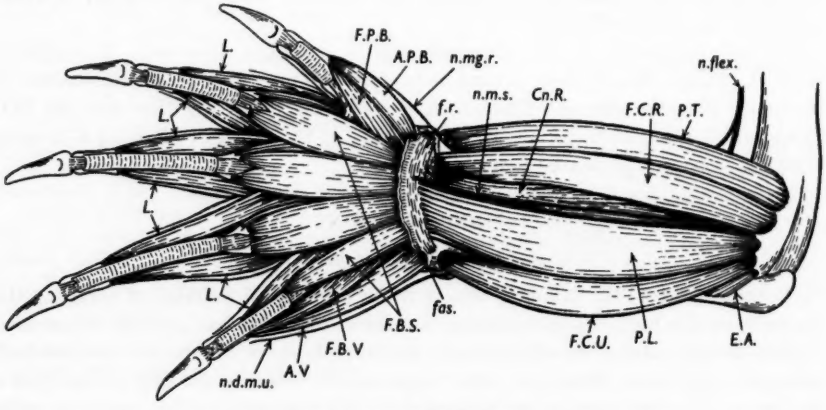

Fig. 1. Superficial muscles.

Figs. 1–4. *Varanus exanthematicus*, dissections of forearm flexors.

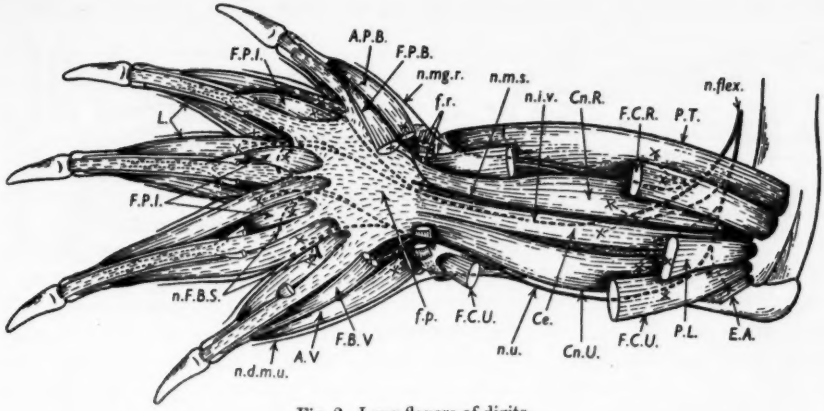

Fig. 2. Long flexors of digits.

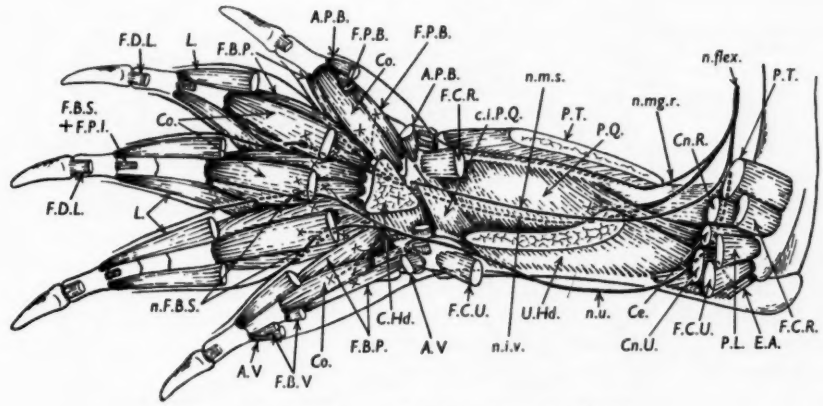

Fig. 3. Pronator layer and deep short muscles of hand.

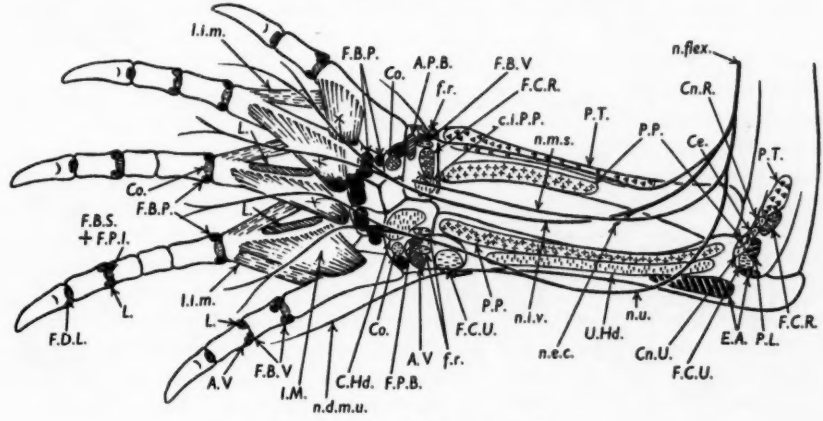

Fig. 4. Bones and intermetacarpals.

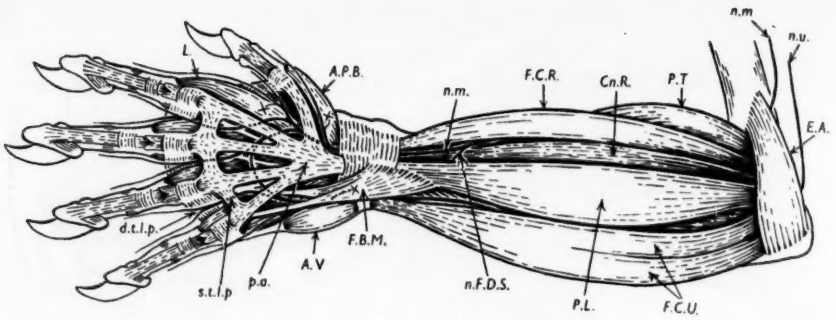

Fig. 5. Superficial muscles.

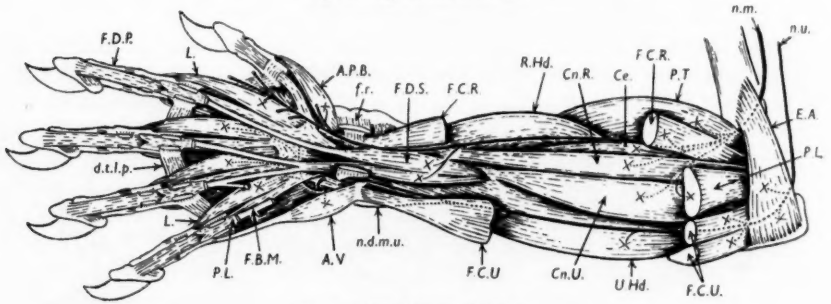

Fig. 6. Long flexors of digits.

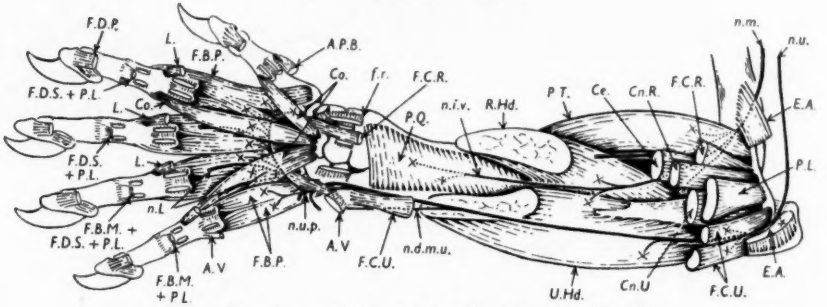

Fig. 7. Pronator quadratus and deep short muscles of hand.

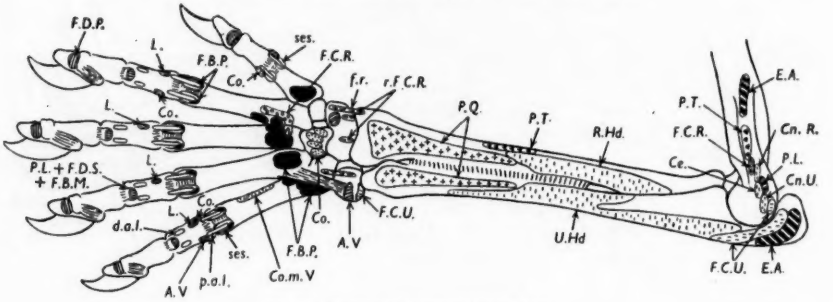

Fig. 8. Bones and muscular origins.

Figs. 5–8. ***Felis domesticus,*** **dissections of forearm flexors.**

In some carnivores and insectivores there may be two separate muscles, a 'palmaris longus externus' and 'internus' (Windle & Parsons, 1897, p. 395; Parsons, 1898, p. 317). Straus (1942, p. 291) has suggested that the palmaris longus may have three possible origins, giving a 'radialis', 'intermedius' and 'ulnaris' derived from the flexor carpi radialis, flexor digitorum sublimis and flexor carpi ulnaris, though not more than two muscles are found in any one animal. But in *Genetta* (Fig. 9E) the doubling is associated with a peculiar double insertion, one slip passing to the palmar aponeurosis and the other to the short muscles of the hand, and it is known that separate insertions may lead to the splitting of a muscle, for example, the separation in man and in *Gorilla* of an extensor pollicis brevis with a phalangeal insertion from the abductor pollicis longus (Straus, 1941, p. 208). In *Cerocoleptes* (Windle & Parsons, 1897, p. 396) there are two muscles supplied by separate nerves, one from the median and the other from the ulnar, but as it is now believed that muscles can acquire new nerve supplies (Haines, 1935; Straus, 1946*a*) this may have no morphological significance. So it seems more probable that the palmaris longus may have become split in some animals than that it has been derived from several different sources.

Flexores breves superficiales

In *Varanus* four short superficial flexors spring from the superficial band of the flexor retinaculum, and, covered only by a thin fascia and skin, pass to digits II–V. Each of those to II, III and IV splits to form a pair of slender tendons which embrace the main flexor tendon, and then enter the fibrous tunnel of the digit and re-unite to be inserted on the penultimate phalanx, while that to V is inserted on the tunnel wall. The abductors and short flexors of the marginal digits appear to belong to this layer (Howell, 1936*a*, p. 306). Abductor I passes from the flexor retinaculum to the basal phalanx, and abductor V from the pisiform to both the basal and middle phalanges. The short flexors both cross the palm, passing from the pisiform to I and from the radiale to V. The main series of superficial flexors is supplied by branches from the common digital branches of the interosseous and ulnar nerves, which pass between the long flexor tendons to reach them; the marginal muscles are supplied by special branches from the median and ulnar nerves.

In *Iguana* (Rabl, 1915) and *Uromastix* (Humphry, 1872, p. 64, 'flexor sublimis digitorum', and personal observations) extra tendons pass to the second phalanx of III and to the third of IV. So the relatively simple arrangement of *Varanus* is not primitive, but depends on the reduction of the phalanges in digits III and IV. Again, in many lizards the short flexors of the marginal digits are not differentiated from the other muscles, so there is a simple series of five short flexors, each giving two slips to its digit, as in *Tejus* (Ribbing, 1907). But all reptilian groups agree in the general arrangements of the superficial layer.

In *Felis* the flexor brevis digitorum manus arises from the anterior surface and ulnar border of the tendon of the palmaris longus, and by an aponeurosis from the radius. It is inserted by two slips to digits IV and V, where it contributes to the formation of the perforated tendons (and sometimes to III as well, Reighard & Jennings, 1929, 'ulnar part of the flexor sublimis digitorum'). It is supplied by a special branch of the deep ulnar nerve. There are well-developed abductors for the

marginal digits, but the short flexors are either absent or represented by a minute muscle crossing the palm to the basal phalanx of I (Reighard & Jennings, fig. 77, '*w*').

Though Windle & Parsons (1897) described and figured the flexor brevis manus quite clearly in several carnivores, it has often been confused, on account of its perforated tendons, with the flexor digitorum sublimis, for example by McMurrich (1903), who figures the muscle arising from the palmaris longus in *Felis* and *Didelphis*. But Ribbing (1907) has described the muscle in a large number of mammals, and he and Straus (1942) leave no doubt as to the individuality of the muscle, or as to its homology with the superficial short muscles of reptiles.

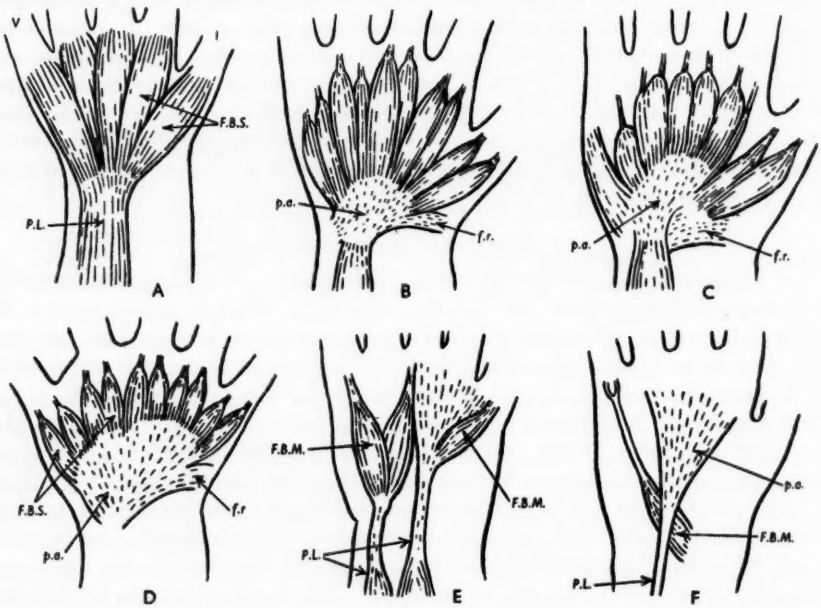

Fig. 9. Flexores breves superficiales and palmar aponeurosis. A, *Tejus*, from Ribbing (1907); B, *Uromastix* (original); C, *Iguana*, from Rabl (1915); D, *Sphenodon*, from Miner (1925); E, *Genetta*, from Windle & Parsons (1897); F, *Herpestes*, from Windle & Parsons (1897).

Palmar aponeurosis

In *Varanus* the superficial short flexors are fleshy throughout the palm, and there is no palmar aponeurosis, a common condition in lizards (Fig. 9A). But in *Uromastix* and *Iguana* the muscles are not attached directly to the superficial band of the retinaculum, but to a palmar aponeurosis (Rabl, 1915, and my Fig. 9B, C), and in *Sphenodon* the aponeurosis is very well developed (Miner, 1925, and my Fig. 9D), *Felis* is highly specialized in having the aponeurosis arranged in five definite slips of which one passes to the flexor tunnel of digit I, while the others, after being joined by cross-connexions, are inserted partly into the flexor tunnels of the digits, but chiefly through the perforated tendons into the middle phalanges. Most mammals have a simple aponeurotic sheet attached to the flexor retinaculum and

palmaris longus proximally, and to the flexor tunnels distally, as in man. The palmaris longus, superficial part of the flexor retinaculum, superficial short flexors and palmar aponeurosis seem always to belong to one superficial formation, but there is great variation in the details of the arrangement of the muscle fibres, and in the proportions of muscular and tendinous tissue (Fig. 9E, F).

Flexor digitorum longus

The main flexor of the digits, the flexor digitorum longus of Rabl (1915); ('flexor palmaris superficialis' and 'profundus' of Eisler (1895), McMurrich (1903), and Straus (1942); deeper parts of the 'flexor primordialis communis' of Ribbing (1907, 1938)) has five distinct heads in *Varanus* (Fig. 2). Three, the condylo-radialis, condylo-ulnaris and centralis of Windle (1889), arise from the humerus, and are separated from each other by the median nerve and the nerve to the palmaris longus. A massive deep head arises from the shaft of the ulna, and a carpal head from the ulnare. The deep head forms a broad, thick flexor plate which receives the attachments of the three humeral heads on its superficial surface just above the wrist, and the carpal head on its deep surface in the palm. Five strong tendons arise from the plate and pass through the fibrous flexor tunnels of the fingers to the terminal phalanges. All the divisions are supplied by branches of the n. flexorius, the carpal head from its anterior interosseous branch. Other lizards agree closely with *Varanus* (Rabl (1915), and Howell (1936*a*), *Iguana*; Straus (1942), *Ctenosaura* and *Scleoporus*), but the detailed structure of the muscle has not been figured in other groups of reptiles.

In *Felis* (Fig. 6) the three humeral heads of the flexor digitorum profundus are distinct, and the nerve to palmaris longus passes between the condylo-radialis and condylo-ulnaris, but it runs superficially to the centralis, not deep to it as in *Varanus*. The deep head of lizards is represented by the ulnar and radial heads of mammals. The carpal head is not developed, but in monotremes, lipotyphlous insectivores (cf. Fig. 10) and ungulates it is represented by a fibrous band (Cambell, 1936, p. 233; Straus, 1942, p. 299). In *Felis* the condylo-ulnaris and centralis join the deep heads near the middle of the forearm, but the condylo-radialis remains separate to the level of the wrist. The insertion is as in *Varanus*. All the heads are supplied by the median nerve, and the ulnar head has an additional branch from the ulnar nerve. Other mammals resemble *Felis* in structure, but the various heads may be lost or coalesce, and in monotremes there is but a single deep head, attached to the ulna as in lizards (Windle, 1889; Straus, 1942).

Paratendinous intravaginal flexors

As the tendons of the superficial short flexors run to their insertions they are joined by a series arising from muscles attached to the flexor plate. These muscles are distinguished by their 'paratendinous' origin (Howell, 1936*a*, p. 306) and intravaginal insertions, for no other muscles have this combination of characters. In *Varanus* there are only three members of the series, each inserted on the radial side of its digit, but in *Iguana* slips pass to both sides of digit IV and sometimes of digits II and III in addition (Rabl, 1915, 'lumbricales sublimes'; Howell, 1936*a*, 'para-

tendinous division of the superficial flexors'); and Ribbing (1907, 'layer 1*b*') has recorded their presence in other lizards. So the perforated tendons, inserted on the penultimate phalanges, are formed partly by the superficial short flexors and partly by these paratendinous intravaginal muscles. The nerve supply arises with that of the superficial short flexors.

In *Felis* the perforated tendons are formed by combinations of the palmaris longus, flexor brevis manus and flexor sublimis, and in digit IV all three muscles take a part. The sublimis is a small muscle which arises from the anterior surface of the flexor digitorum profundus, passes deep to the retinaculum, and is inserted into digits II–IV. It is supplied by a special branch of the median nerve which crosses the condylo-radialis to reach it. This type of muscle, found in *Felis*, poorly developed

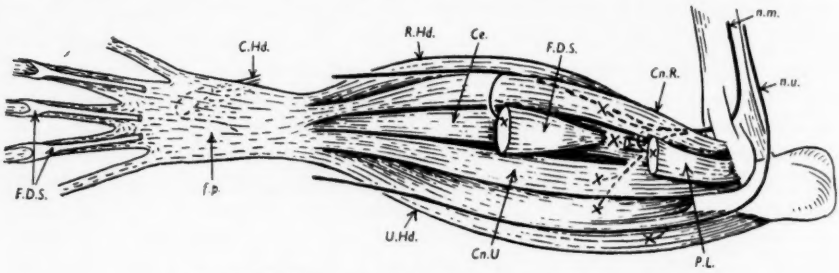

Fig. 10. *Erinaceus aegyptiacus*, long flexors of digits.

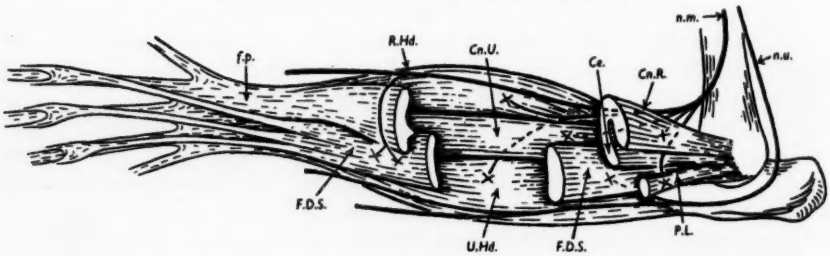

Fig. 11. *Oryctolagus cuniculus*, long flexors of digits.

and with no separate origin from the humerus, is also found in many other carnivores, in marsupials and in monotremes (McMurrich, 1903; Howell, 1936*b*). In *Erinaceus* (Fig. 10) the muscle arises from the humerus, but the belly is small and situated distally in the forearm, and the muscle still has its peculiar nerve supply.

In *Oryctolagus* (Fig. 11) the sublimis is a large, powerful muscle with a fleshy origin from the humerus and a new proximal nerve supply from the ulnar nerve, but it possesses in addition the old distal supply from the median. The distal supply crosses superficial to a large muscle identified by Windle (1889) as a condylo-radialis, but since it has two distinct origins from the humerus, and since the nerve to palmaris longus has its characteristic course between these two heads, the muscle is probably a conjoined condylo-radialis and centralis. In primates, such as man, the muscle has become more important still, with an insertion into four digits instead

of three, a concentration of the fleshy belly near the elbow, and a total loss of the distal nerve supply, but the new supply comes from the median and not from the ulnar nerve. In *Tupaia* the muscle resembles that of the primates but is supplied by the ulnar nerve (Straus, 1942, p. 310).

Assuming, with McMurrich (1903), that the type of muscle found in *Felis* and *Didelphys* is primitive for mammals, and comparing this directly with the paratendinous intravaginal flexors of lizards, they agree in their origins from the main flexor tendons, their insertions through the perforated tendons on the subterminal phalanges, and their positions between the superficial short flexors and palmar aponeurosis on the one hand, and the main flexor tendons on the other. The mammalian muscle is better developed, more concentrated, lies more proximally, and has a nerve supply arising higher up the forearm, but these tendencies all seem to be progressive, finding their highest expression in primates. So it is suggested that the flexor sublimis of the *Felis* type is derived directly from the paratendinous-intravaginal flexors of reptiles, or possibly that both have been derived from the same structure in some ancestral form.

How the humeral origin found in most mammals was achieved is unknown, for detailed information about the muscle is not yet available. Windle & Parsons' (1897) Table shows that it may be associated with any one of the three humeral heads of the profundus, and Straus (1942, p. 303) takes the view that one or more of these heads may be incorporated in it. But since a separate humeral origin may co-exist with a complete set of humeral heads, as in *Erinaceus*, it is equally possible that the muscle has merely extended its origin as far as the humerus, and that it may be partly fused with one or other of the other muscles attached there.

Eisler (1895), looking at the reptilian perforated tendon inserted into the subterminal phalanx, and the mammalian perforated tendon, considered them homologous structures, a conclusion firmly based on a close morphological similarity. But at that time the reptilian paratendinous intravaginal flexors were inadequately known, so that the tendons were considered the exclusive property of the superficial short flexors, and similarly in mammals the importance of the flexor brevis manus was not appreciated, and in them the perforated tendon was considered the property of the sublimis. This led to an attempt to homologize, in part or in whole, the superficial short flexors of reptiles with the sublimis of mammals, a solution which offered many difficulties, and which was finally discarded by Straus (1942, p. 303). For, as he pointed out, it is the flexor brevis manus that is the mammalian equivalent of the superficial short flexors of reptiles.

Howell (1936*a*) has divided the flexor muscles rather strictly into brachial, antibrachial and podial groups, and though he believed a muscle might migrate from one region to another he looked for the homologue of the sublimis in the reptilian forearm. So he suggested that the sublimis has, in mammals, become split off from the humeral heads of the flexor digitorum longus as a completely new muscle with its own, newly formed, set of tendons (p. 305). But there is little evidence in support of this suggestion, and it does violence to the obvious homology between the perforated tendons of reptiles and mammals.

The theory put forward here was foreshadowed by McMurrich (1903), who derived the mammalian sublimis and the flexor brevis manus, which he confused with it,

from his 'flexores breves superficiales', of which he figured, in the lizard *Liolepisma* (his Fig. 3), two layers, a superficial and a deep, corresponding to my superficial short flexors and paratendinous intravaginal flexors. But this aspect of his work, being poorly illustrated, mostly by transverse sections, is difficult to understand, and has not been appreciated. In essence the theory follows Humphry's subdivision of the long flexors of mammals into three layers, the palmaris longus, sublimis and profundus, used by Windle & Parsons (1897, p. 72) as a basis for their work, but suggests that though in reptiles the first and third layers are represented by the palmaris and flexor digitorum longus, the middle layer is formed by a few short muscles originally confined to the hand. It is in fundamental disagreement with the basic plan suggested by Howell (1936*a*, p. 304), followed by Straus (1942, p. 282), based on anomalous specimens of *Necturus*, that the humeral and profundus heads of the flexor digitorum longus are derived from different 'matrices' that have become fused to form a composite muscle in reptiles, but that this union has broken down in mammals to give a sublimis and profundus.

Lumbricals

A group of seven muscles is inserted, in *Varanus*, on the bases of the subterminal phalanges outside the fibrous sheaths. Five of these arise from the distal margin and deep surface of the flexor plate, and the other two from metacarpals III and IV. These muscles, McMurrich's (1903) 'superficial layer of the flexor brevis', Rabl's (1915) 'lumbricales profundi', are universally recognized as the homologues of the mammalian lumbricals, which are also extravaginal in position, but are only developed on the radial sides of the digits. In *Varanus* they are supplied by the common digital nerves, in *Felis* that to digit II is supplied by a branch of the median, and the others by the deep ulnar nerve (Reighard & Jennings (1929, p. 392), gave the nerve supply of the three radial muscles as probably from the median nerve).

Contrahentes

In *Varanus* a group of five short muscles is inserted into the bases of the proximal phalanges, those to digits I and V arising from the ulnare and carpale I, and those to II, III and IV from the deep surface of the flexor plate. In *Sphenodon* and *Iguana*, Miner (1925) and Howell (1936*a*) have recorded as contrahentes only the marginal slips to I and V, but the slips to II, III and IV appear to be true contrahentes in spite of their peculiar origin. In *Felis* the layer includes the contrahentes I, II and V, all taking origin from the capitate, and there is, in addition, a muscle inserted into metacarpal V, the 'M. opponens (adductor) digiti quinti' of Reighard & Jennings (1929), which appears to belong to the series.

Flexores breves profundi and intermetacarpales

In *Varanus* each digit is provided with deep flexors which arise in pairs from the carpal bones, and are inserted on each side of the proximal phalanges. Deep to these lie the intermetacarpal muscles, each of which passes distally and radially from the base of one metacarpal to the shaft of an adjoining bone (similar in *Iguana*, Howell, 1936*a*, p. 309). Deep to these again are the interosseous ligaments which

pass from the metacarpals through the joint capsules to the bases of the proximal phalanges, two converging on digit IV, and the others passing to II and III. The muscles are supplied by branches of the common digital nerves, which run in the substance of the short flexor muscles. The ligaments are found in other lizards (McMurrich, 1903, Fig. 5, *Iguana*), and also in *Sphenodon*, where Miner (1925) described them as homologous with the intermetacarpal muscles of other animals. But since in *Varanus* the ligaments and muscles co-exist they must be considered as separate structures, though they may belong to the same formation.

In *Felis* the deep short flexors are well developed in digits II–IV. They arise in pairs from the metacarpal bones of their own and of adjacent digits. Each belly splits into two slips, one of which is inserted on the corresponding sesamoid bone of the metacarpo-phalangeal joint, and the other into the extensor tendon on the back of the digit. They are supplied by the deep branch of the ulnar nerve, which passes across the palm partly embedded in the muscular fibres. In primates these muscles form the 'dorsal' and 'volar' interossei.

Intermetacarpals are not developed in *Felis*, but the peculiar 'interossei dorsales' of marsupials and some other mammals may represent this layer, for they lie dorsal to, and may co-exist with, a complete series of deep flexors. The contrahentes and deep flexors have been adequately discussed by Forster (1916) with full references to the literature.

Flexor retinaculum

In *Varanus* the flexor retinaculum consists of two quite separate bands, a superficial and a deep, both arching from the radiale to the pisiform. The superficial band is convex distally, where it gives origin to the slips of the flexor brevis superficialis, and concave proximally, where it receives the palmaris longus, but the deep head is free of muscular attachments. Between the two bands pass the whole of the ulnar nerve, and the deep palmar branch of the median nerve on its way to supply the superficial thenar muscles, and the bands are also separated by the origins of the flexor brevis pollicis and flexor brevis V. The deep band is found in other lizards, but the superficial is often incomplete, with no attachment to the radiale, a condition figured by Ribbing (1907) in *Tejus* (my Fig. 9A). In *Felis* the retinaculum is single, but has the special retinaculum for the palmaris longus attached to its superficial surface. The median nerve runs deep and the ulnar nerve superficial to it.

Pronator profundus

In *Varanus* the pronator profundus slopes distally from the ulna to the radius, crossing the wide interosseous space, and extra slips are attached to the humerus and radiale. It is a powerful muscle concerned with pronation, whose range is about 60° in this genus (Haines, 1946). Other reptiles have a similar layer of muscle, usually with the extra slips (Straus, 1942, p. 301). In *Felis*, where pronation is exceptionally free (about 90°), the corresponding muscle, the pronator quadratus, is better developed than in most mammals, but it is still small compared with the reptilian muscle. Probably the greater part is concerned with the formation of the interosseous ligament ('membrane'), an exclusively mammalian structure (Haines, 1946). It has been suggested, too, that the deep head of the pronator teres, in those

animals in which it exists, belongs to this layer, but Straus (1942, p. 286) has shown that this is unlikely. The difference in relationship of the muscle to the anterior interosseous nerve (superficial in lizards, deep in mammals), adds to the strength of Straus's contention.

DISCUSSION

Relationship of lizards and mammals

The lizards are one of the surviving groups of reptiles with limbs, and together with *Sphenodon*, to which they seem closely related, may well be more primitive in the arrangement of their limbs than the other groups, the turtles and crocodiles, which have specialized gaits. But it is hardly to be expected that they retain absolutely unchanged the limb structure of the ancestral stem-group from which the mammalian stem also sprang. So, though a closely similar arrangement may be found in living reptiles and mammals, it is not safe to assume that it was inherited as such from a common ancestral type. Indeed, in some points which can be tested by appeal to the fossil series, it can be demonstrated that both groups have undergone the same changes, by parallel evolution, as, for instance, fusion of the radiale and intermedium in *Varanus* (scapho-lunar of many mammals), dwarfing or loss of phalanges in digits III and IV, development of sesamoids (Haines, 1940), formation of secondary epiphysial centres and the development of cartilage canals in the genus *Varanus* (Haines, 1942). But in the case of the muscles, even if many of the similarities in lizards and mammals are due to parallel evolution, there is no reason to believe that the primitive arrangements in their common ancestors were very different.

Tendons, aponeuroses and retinacula

In discussing the palmar aponeurosis and flexor tendons of the hand Howell (1936*a*, p. 301) remarks that 'Fascia is an all-pervading connective tissue. Tenuous sheets of it can become tougher in any situation, and subdivide into layers'. His 'own belief is that only slight reliance can be placed upon the fascial arrangements in the diverse groups of vertebrates'. Straus (1942, p. 303) suggests that 'tendon actually being no more than specially orientated collagenous tissue, it is quite conceivable that, given the proper tensile stimulus, it readily might develop from elements that otherwise would form looser fascia'. The opposite point of view, that tendons, aponeuroses and retinacula are rather definite structures, closely connected with the muscular system and not mere condensations of the general fascia, was supported by Bland-Sutton (1920), and a detailed study of the forearm and hand supports his views. The palmar aponeurosis is seen to be formed, not from a condensation of the 'thin fascia superficial to the long flexor tendons and flexores superficiales' (Straus, 1942, p. 304), but from the superficial short flexors themselves. The tendons of the flexor digitorum sublimis are seen to exist as tendons in reptiles, and not to have been 'formed de novo from adjoining mesenchyme' (Straus, p. 303).

Again, it is doubtful if the transverse carpal ligament can 'represent superficial fascia' (Howell, 1936*a*, p. 307). Embryologically a muscle, its tendon, its tendon-sheath, and any retinacula associated with it, are all developed from a single pre-muscle mass, and the parts only become distinguishable from each other later on. For example, at 20 mm. the rectus abdominis and rectus sheath form a single

compact mass, isolated from other structures by loose mesenchyme (Walmsley, 1937). The long flexor tendons, and their fibrous tunnels with the vaginal ligaments first appear as one homogeneous mass, though later the tissue between the tendons and fibrous tunnels thins to form synovial mesenchyme, and eventually breaks down to form a cavity (Retterer, 1896). So, though the development of the retinaculum of the wrist has not been studied in detail, it seems reasonable to suggest that it is associated morphologically with the muscles, the superficial band of the palmaris longus and superficial short flexors which are attached to it, and the deep band with the long flexor tendons which it binds in place.

New muscles

Straus (1942, p. 312) has called attention to the close correspondence of mammalian and reptilian forearm muscles, and has prepared a detailed table of their homologies. He found only two, the palmaris longus and flexor digitorum sublimis, that could be claimed as 'distinctly new' in mammals. But if, as is suggested in this paper, the palmaris longus is an old muscle and the flexor digitorum sublimis is represented by a set of short hand-muscles in reptiles, then there are no new muscles at all in the mammalian forearm. In the hand Straus (1946*b*) has suggested that 'muscular homologies, at least between vertebrate classes, cannot be reasonably extended beyond comparison of entire palmar layers. Direct homology of individual muscle units is profitless and probably invalid.' But it seems, on general grounds, unlikely that the forearm and hand should differ so radically that homologies can be established in the one region and not in the other, and in practice it has been found possible to compare individual muscles. In mammals no completely new muscles seem to have appeared, though several slips have been lost, including the flexors of the lost phalanges of digits III and IV, the three lumbricals attached to the ulnar sides of the digits, and the set of intermetacarpal muscles.

Functions of muscles

Several muscles pass from the humerus to the forearm bones, particularly to the radius, including the tractor radii, extensor radialis intermedius and profundus, and extensor ulnaris (Haines, 1939), and the pronator teres and epitrochleo-anconeus. Now Howell (1936*a*, p. 291) suggests that 'muscles passing from the condyle of the humerus to the forearm can do nothing but fix the joint', and that ' to be actually useful the attachment must shift in certain ways', and Straus (1942, p. 294) that the epitrochleo-anconeus 'is capable of little more than fixation of the elbow joint'. But in view of the extensive movements of the forearm bones in relation to the humerus both in flexion-extension and in pronation-supination (in reptiles both bones rotate on the humerus, Haines, 1946), it is difficult to accept this view. The pronator teres is a powerful flexor and pronator, and the epitrochleo-anconeus helps to rock the ulna medially in supination, a function recognized in mammals by Hultkrantz (1897), who correlated the absence of the muscle in man with a loss of ability to rock the ulna on the trochlear surface of the humerus.

Nerves

In *Varanus* all the nerves supplying the flexores of the forearm are derived from a single trunk, the n. flexorius of Ribbing (1907) or n. brachialis longus inferior of Osawa (1898). This trunk (Fig. 12E, *X*) breaks up into four divisions, all of which enter the forearm by passing deep to the pronator teres. The marginalis radialis reaches the hand by passing between the pronator teres and flexor carpi radialis, both of which it supplies. The medianus superficialis passes between the condyloradialis and centralis to reach the skin of the palm to supply two thenar muscles, but it does not supply the digits. The interosseus volaris passes deep to the pronator

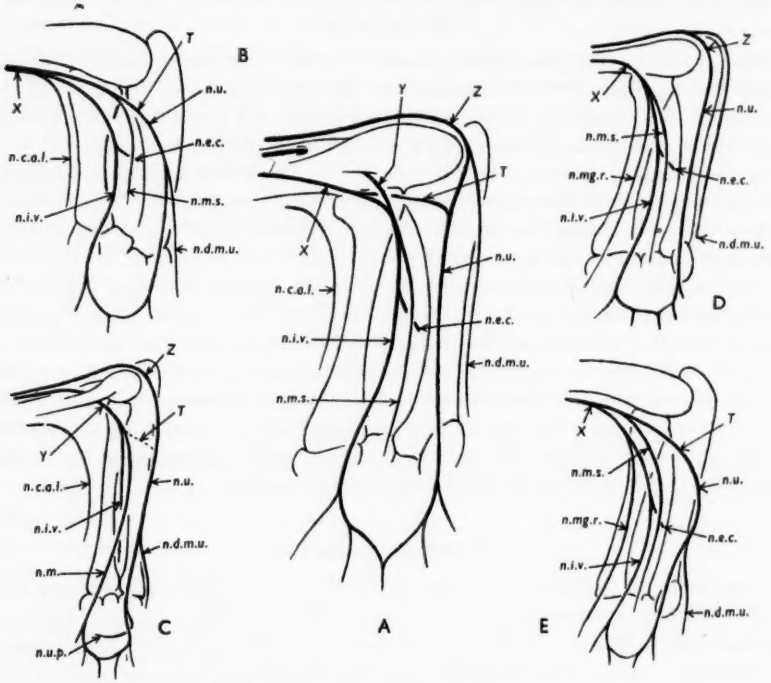

Fig. 12. Nerve trunks of the forearm. A, *Sphenodon*; B, urodele; C, mammal; D, lacertid; E, varanid.

quadratus, and after giving off a large extensorius communicans, which passes through the interosseous space to supply the extensor muscles and back of the hand (Haines, 1939, Fig. 8), continues to the palm, where it supplies palmar muscles and the more radial digits. The ulnaris (*T*) passes transversely across the upper part of the forearm deep to the humeral heads of the flexor digitorum longus, and then turns towards the hand under cover of the flexor carpi ulnaris. Above the wrist it gives off a dorsalis manus ulnaris to the ulnar border of the hand, and then passes into the palm to supply muscles and ulnar digits, forming, with the interosseus volaris, a system which lies deep to the flexor tendon plate. The arrangement is similar in

Ameiva, *Tupinambis* and *Teguexin* (Ribbing, 1907). In many lizards, however, such as *Lacerta* (Ribbing, 1907), *Iguana* (Howell, 1936*b*), *Sceloporus* and *Ctenosaura* (Straus, 1942), the ulnar nerve separates from the others above the elbow, and passes, as it does in mammals, behind the medial epicondyle of the humerus (Fig. 12D, *Z*), though, apart from this difference of origin and the higher separation of the n. dorsalis manus ulnaris, the nerve trunks behave as in *Varanus*.

Now Ribbing, since he found the varanid arrangement, with a single path of entry, in urodele amphibians and in the turtle *Emys*, considered it as primitive, and the lacertid arrangement as derived from it by the shift of the ulnar fibres behind the epicondyle. But in *Sphenodon* (Osawa, 1898) there are three routes by which flexor nerves enter the forearm, one anterior to the epicondyle, the n. humeralis superior (Fig. 12A, *X*), one through the entepicondylar foramen, the n. humeralis inferior (*Y*), and the third behind the epicondyle, the n. ulnaris (*Z*). Pathway *X* gives a transverse communicating branch (*T*) to the ulnar, and then joins *Y* to form a trunk which soon breaks up into the medianus superficialis and interosseus volaris. A separate marginalis radialis is not recorded, but otherwise the nerve trunks behave as in lizards. The great majority of early tetrapods possessed an entepicondylar foramen (found in living animals only in *Sphenodon* and in some mammals), and the presence of the foramen implies that of a nerve trunk. So it is probable that the complex arrangement in *Sphenodon* is more primitive than the relatively simple arrangement found in other living types. A similar secondary simplification of the nervous pathways is also found in the extensor region of the forearm in lizards (Haines, 1939).

In mammals, all the fibres of the median nerve usually pass through the entepicondylar foramen, and the n. extensorius communicans has been lost, probably on account of the approximation of the radius and ulna and the development of a powerful interosseous ligament between them (Haines, 1946). The n. medianus superficialis, the 'median' nerve of mammals, passes distally between the flexor carpi radialis and condylo-radialis instead of between the condylo-radialis and centralis. Pathway *T* is usually lost, but may be represented by the transverse communication found in *Gorilla* and other primates (Straus, 1942, p. 310). Otherwise the arrangement of the nerve trunks, and even the details of the muscular supplies, have remained remarkably constant. In the hand, a new superficial nervous arch has been formed between the medianus superficialis and the ulnaris, and this arch supplies the digits, while the older deep arch is represented by the 'deep branch of the ulnar', which supplies only muscles and joints. The reason for these changes, discussed by Straus (1942), is unknown.

SUMMARY

1. The muscles and nerves of the flexor region of the forearm and hand are mapped in detail for the lizard *Varanus* and the mammal *Felis*.

2. The muscles correspond very closely in lizards and mammals; none have been added in mammals, though a few short hand-muscles have been lost.

3. The pronator teres, flexor carpi radialis, palmaris longus, flexor carpi ulnaris and epitrochleo-anconeus are found as such in reptiles, the flexor digitorum pro-

fundus is represented by the flexor digitorum longus, and the pronator quadratus by the pronator profundus.

4. The flexor digitorum sublimis of mammals is represented in reptiles by a series of short hand-muscles which arise from the tendon plate of the flexor digitorum longus, and are inserted within the flexor sheaths of the digits. The muscle has migrated proximally in mammals, acquiring new nerve supplies in its progress.

5. The course of the fibres of the median and ulnar nerves is variable in reptiles and urodeles. Probably *Sphenodon* presents a primitive condition, with roots passing in front of the medial epicondyle of the humerus, through the entepicondylar foramen and behind the epicondyle.

6. The palmar aponeurosis, flexor retinaculum and tendons of the flexor digitorum sublimis are all represented as such in reptiles, and have not been formed *de novo* from condensations of loose connective tissue.

REFERENCES

Bland-Sutton, J. (1920). *Selected Lectures and Essays*, 7th ed. London: Heinemann.

Cambell, B. (1936). The comparative myology of the forelimb of the hippopotamus, pig and tapir. *Amer. J. Anat.* **59**, 201–247.

Eisler, P. (1895). Die Homologie der Extremitäten. *Abh. naturf. Ges. Halle*, **19**, 87–346.

Forster, A. (1916). Die Mm. contrahentes und interossei manus in der Säugetierreihe und beim Menschen. *Arch. Anat. Physiol., Lpz.*, 101–378.

Haines, R. W. (1935). A consideration of the constancy of muscular nerve supply. *J. Anat., Lond.*, **70**, 33–55.

Haines, R. W. (1939). A revision of the extensor muscles of the forearm in tetrapods. *J. Anat., Lond.*, **73**, 211–233.

Haines, R. W. (1940). A note on the independence of sesamoid, intratendinous and epiphysial centres. *J. Anat., Lond.*, **75**, 101–105.

Haines, R. W. (1942). The evolution of epiphyses and of endochondral bone. *Biol. Rev.* **17**, 267–292.

Haines, R. W. (1946). A revision of the movements of the forearm in tetrapods. *J. Anat., Lond.*, **80**, 1–11.

Howell, A. B. (1936*a*). Phylogeny of the distal musculature of the pectoral appendage. *J. Morph.*, **60**, 287–315.

Howell, A. B. (1936*b*). The musculature of antibrachium and manus in the platypus. *Amer. J. Anat.* **59**, 425–432.

Hultkrantz, J. W. (1897). *Das Ellbogengelenk und seine Mechanik.* Jena: Fischer.

Humphry, G. M. (1872). *Observations in Myology.* Cambridge and London: Macmillan.

McMurrich, J. P. (1903). The phylogeny of the forearm flexors. *Amer. J. Anat.* **2**, 177–209.

Miner, R. W. (1925). The pectoral limb of Eryops and other primitive tetrapods. *Bull. Amer. Mus. nat. Hist.* **51**, 145–312.

Osawa, G. (1898). Beiträge zur Anatomie der *Hatteria punctata*. *Arch. mikr. Anat.* **51**, 481–690.

Parsons, F. G. (1898). The limb myology of *Gymnura rafflesii*. *J. Anat., Lond.*, **32**, 312–324.

Rabl, C. (1915). Über die Muskeln und Nerven der Extremitäten von *Iguana tuberculata* Gray. *Arb. anat. Inst., Wiesbaden*, **53**, 681–789.

Reighard, J. & Jennings, H. S. (1929). *Anatomy of the Cat*, 2nd ed. New York: Holt.

Retterer, E. (1896). Sur le développement morphologique et histologique des ourses muqueuses et cavités péri-tendineuses. *J. Anat. Physiol., Paris*, **32**, 256–300.

Ribbing, L. (1907). Die distale Armmuskulatur der Amphibien, Reptilien und Säugetiere. *Zool. Jb.* (*Abt.* **2**, *Anat. Ontog.*), **23**, 587–682.

Ribbing, L. (1938). *Die Muskeln und Nerven der Extremitäten.* In: Bolk, Göppert, Kallius & Lubosch, *Handb. vergleich. Anat. Wirbeltiere.* Berlin: Urban and Schwarzenberg.

Straus, W. L., Jr. (1941). The phylogeny of the human forearm extensors. *Hum. Biol.* **13**, 23–50, 203–238.

Straus, W. L., Jr. (1942). The homologies of the forearm flexors: urodeles, lizards, mammals. *Amer. J. Anat.* **70**, 281–316.

Straus, W. L., Jr. (1946*a*). The concept of nerve-muscle specificity. *Biol. Rev.* **21**, 75–91.

STRAUS, W. L., Jr. (1946*b*). The pattern of the intrinsic palmar musculature. *Biol. Bull. Wood's Hole.* **91**, 233.

WALMSLEY, R. (1937). The sheath of the rectus abdominis. *J. Anat., Lond.*, **71**, 404–414.

WINDLE, B. C. A. (1889). The flexors of the digits of the hand. I. The muscular masses in the forearm. *J. Anat., Lond.*, **24**, 72–84.

WINDLE, B. C. A. & PARSONS, F. G. (1897). On the myology of the terrestrial carnivora. *Proc. zool. Soc., Lond.*, pp. 370–409.

KEY TO LETTERING

A.P.B.	abductor pollicis brevis
A. V	abductor digiti V
Ce.	centrale
C.Hd.	carpal head
c.i.P.P.	carpal insertion of pronator profundus
c.i.P.Q.	carpal insertion of pronator quadratus
Co.	contrahens
Co.m. V	contrahens metacarpi V
Cn.R.	condylo-radialis
Cn.U.	condylo-ulnaris
d.a.l.	distal annular ligament
d.t.l.p.	deep transverse ligament of palm
E.A.	epitrochleo-anconeus
fas.	fascia
F.B. V	flexor brevis digiti V
F.B.M.	flexor brevis manus
F.B.P.	flexor brevis profundus
F.B.S.	flexor brevis sublimis
F.C.R.	flexor carpi radialis
F.C.U.	flexor carpi ulnaris
F.D.L.	flexor digitorum longus
F.D.P.	flexor digitorum profundus
F.D.S.	flexor digitorum sublimis
f.p.	flexor plate
F.P.B.	flexor pollicis brevis
F.P.I.	paratendinous intravaginal flexor
f.r.	flexor retinaculum
I.M.	intermetacarpal
L.	lumbrical
l.i.m.	intermetacarpal ligament
n.c.a.l.	n. cutaneus antibrachii lateralis
n.d.m.u.	n. dorsalis manus ulnaris
n.e.c.	n. extensorius communicans
n.F.B.S.	n. to flexor brevis superficialis
n.F.D.S.	n. to flexor digitorum sublimis
n.flex.	n. flexorius
n.i.v.	n. interosseus volaris
n.L.	n. to lumbrical
n.m.	n. medianus
n.mg.r.	n. marginalis radialis
n.m.s.	n. medianus superficialis
n.u.	n. ulnaris
n.u.p.	n. ulnaris profundus
p.a.	palmar aponeurosis
p.a.l.	proximal annular ligament
P.L.	palmaris longus
P.P.	pronator profundus
P.Q.	pronator quadratus
P.T.	pronator teres
r.F.C.R.	retinaculum for flexor carpi radialis
R.Hd.	radial head
ses.	sesamoid
s.t.l.p.	superficial transverse ligament of palm
U.Hd.	ulnar head

T, *X*, *Y*, *Z* in fig. 12, see text.

DIFFERENTIATION OF MAMMALIAN EPIDERMIS IN TISSUE CULTURE

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The normal histogenesis of the epidermis in rats and mice was studied *in vitro*, with a view to investigating the effects on the skin of various physical and chemical agents (radiations, carcinogens, hormones, vitamins, etc.). Though the research had to be discontinued, the purpose of the present note is to show that the epidermis of embryonic rats does develop satisfactorily in tissue culture and should be useful material for experiments of the type indicated above.

Drew (1922) briefly recorded keratinization in cover-slip cultures of epidermis explanted from embryonic mice. Maximow (1925) studied the histogenesis of various tissues in fragments of rabbit embryos grown in cover-slip cultures; he found abnormalities in keratinization, although the early development of the epidermis was more normal. Hiraiwa (1927) grafted portions of rat embryos on the chorio-allantoic membranes of chicks, and reported normal epidermal development and keratinization. Chlopin (1932) has described atypical keratinization of human embryonic epidermis in cover-slip cultures. Strangeways & Fell (1926) also reported slightly abnormal keratinization of the epidermis in chick limb buds cultivated in centrifuge tubes. The most detailed account of epidermal histogenesis *in vitro* is that of Miszurski (1937), who grew fragments of chick limb buds on cover-slips, and whole limb buds in watch-glasses. In the cover-slip cultures connective tissue grew round the epidermis, which formed cysts, and keratinized normally. Litvac (1939) confirmed these observations. Miszurski found abnormal keratinization in the watch-glass cultures where the epidermis remained as a covering to the mesodermal tissues of the limb bud.

In the present experiments most of the cultures were made from 12-day rat embryos, some from 16-day rat embryos, and a few from 16-day mouse embryos. The epidermis, with some underlying mesoderm, was taken from several parts of the body: anterior dorsal, anterior ventral, posterior dorsal, tail, and forelimb. The tissues were grown on cover-slips inverted over hollow slides. The culture medium consisted of a mixture of equal parts of fowl plasma and of extract of chick embryos in Pannet and Compton solution. At 2-, 3-, 4- or 5-day intervals, the piece of explanted tissue was cut away from its surrounding outgrowth and transferred to a fresh drop of culture medium. Transference every 2 or 3 days appeared to be the most satisfactory. No attempt was made to maintain the explanted tissues for very long periods, but one fragment appeared to be healthy after 30 days' cultivation.

Observations were made on serially sectioned explants. During the first few days *in vitro* the edges of the explant curl up and meet to form a hollow cyst, of which the original outer surface of the epidermis forms the lining. As differentiation proceeds this cyst becomes filled with keratin.

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The epidermis of a 12-day rat embryo consists of a single layer of flattened cells (Hanson, 1947). *In vitro*, as *in vivo*, this layer produces a periderm, becomes several layers thick, and then, as its cells differentiate, develops the stratification typical of rat epidermis. Epidermis which has been grown *in vitro* appears to differ from the normal only in the formation of a cyst and in the persistence of the periderm. Development *in vitro* is sometimes slower, sometimes rather faster than *in vivo*, and the rate of differentiation varies amongst cultures of the same age and parentage grown in similar conditions.

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REFERENCES

Chlopin, N. G. (1932). Über einige Wachstums- und Differenzierungserscheinungen an der embryonalen menschlichen Epidermis im Explantat. *Arch. EntwMech. Org.* **126**, 69–89.

Drew, A. H. (1922). A comparative study of normal and malignant tissues grown in artificial culture. *Brit. J. exp. Path.* **3**, 20–27.

Hanson, J. (1947). The histogenesis of the epidermis in the rat and mouse. *J. Anat., Lond.*, **81**, 174–197.

Hiraiwa, Y. K. (1927). Studies on grafts of embryonic tissues of the rat on the chorio-allantoic membrane of the chick. I. Differentiation of ectodermal derivatives. *J. exp. Zool.* **49**, 441–457.

Litvac, A. (1939). Sur la kératinisation épithéliale *in vitro*. *Arch. Anat. micr.* **35**, 55–63.

Maximow, A. (1925). Tissue-cultures of young mammalian embryos. *Contr. Embryol. Carneg. Instn*, **16**, 49–113.

Miszurski, B. (1937). Researches on the keratinisation of the epithelium in tissue-cultures. *Arch. exp. Zellforsch.* **20**, 122–139.

Strangeways, T. S. P. & Fell, H. B. (1926). Experimental studies on the differentiation of embryonic tissues growing *in vivo* and *in vitro*. I. The development of the undifferentiated limb-bud (*a*) when subcutaneously grafted into the post-embryonic chick, and (*b*) when cultivated *in vitro*. *Proc. roy. Soc.* B, **99**, 340–366.

HISTOCHEMICAL CHANGES PRODUCED BY COLD AND ADRENALINE IN THE SUPRARENAL CORTEX OF THE ADULT MALE RAT

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THE EFFECT OF COLD

Introduction

As early as 1914, Elliott reported that adrenalectomized cats survived longest if kept in a warmed animal house. Later work on the effect of cold on the adrenal cortex (Flexner & Grollman, 1939; Dosne & Dalton, 1941; Levin, 1945; Hemphill & Reiss, 1947) has yielded contradictory results. The present experiments were undertaken to study further the histochemical changes in the adrenal cortex of rats exposed to low temperatures.

Experimental details

This work has been performed on 176 male adult white rats of the Wistar strain of approximately 200 g. weight. All the rats were isolated in separate cages for 7–10 days prior to the exposure to cold, in order to eliminate the possibility of adaptation syndrome phenomena due to contact excitement or sexual stress. Such an isolated male rat was taken as the 'standard' rat of the experiments, though during the course of the work it became evident that the adrenal glands, even in the standard rat, showed considerable variation.

These isolated rats were placed in the refrigerator at 0° C., each in a separate small cage, and were given a standard diet and adequate water throughout the experiment. The cages were small in order to restrict movement which might have raised the animals' temperature. All animals were killed quickly at the end of the experiment by exposure to coal gas. This mode of death was found to produce no discernible histochemical change in the adrenals; the point was investigated in a series of twenty-four rats, twelve of which were killed by coal gas and twelve by a blow on the head.

The adrenal glands were fixed in formol calcium and subsequently stained by Sudan black and by dinitrophenyl hydrazine hydrochloride and were subjected to the Schultz test and the plasmal reaction. Full details of these histochemical tests have previously been given (Yoffey & Baxter, 1949).

In general, the picture given by all four reactions was the same, but the Schultz test was found to be more sensitive and precise than the others. In consequence more reliance has been placed upon it than on the other three histochemical tests. The Schultz reaction is all the more interesting since it is a modification of the Lieberman-Burchardt test for cholesterol.

Twelve isolated rats were killed and their adrenals examined histochemically.

* Aided by a grant from the Colston Research Fund.

In the adrenals of the twelve above-mentioned isolated rats killed by coal-gas it was found that, within the limits of the variation already noted, a fairly definite histochemical picture could be identified, and it served as a normal standard by which changes could be assessed.

Figs. 1–3, Pl. 1, are photomicrographs in black and white of the Schultz reaction in three of these standard rats, and fig. 1, Pl. 2, is another of these in colour.

Four series of experiments were carried out:

Series 1. To discover how short an exposure to 0° C. will produce a demonstrable histochemical change in the adrenal cortex, thirty rats were kept in the refrigerator for times varying from 5 min. to 3 hr. and then killed immediately. After an exposure of 5 min. one can detect a slight degree of cholesterol depletion from the zona reticularis and zona fascicularis as shown in fig. 2, Pl. 2, whilst after 15 min. at 0° C. the depletion is even more marked as shown in fig. 3, Pl. 2.

Series 2. How soon after exposure to cold does the gland return to normal? To determine this, twenty rats were exposed to cold for 3 hr. and killed subsequently at varying intervals. It was found that 72 hr. after exposure the gland was indistinguishable from the normal (fig. 4, Pl. 2).

Series 3. Can cold as a stimulus produce the three stages of Selye's adaptation syndrome? Twenty-six rats were exposed to cold for periods of time varying from 3 hr. up to 1 week. Fig. 4, Pl. 1, shows the Schultz reaction in the gland of a rat exposed to cold for 48 hr.; the cortex with its cholesterol depletion is typical of the *stage of alarm*. Fig. 5, Pl. 1, demonstrates the change to the *stage of resistance*, with the re-accumulation of Schultz-positive material in the inner zone, when a rat is exposed to 0° C. for 72 hr. Fig. 6, Pl. 1, shows a cortex in the third stage, that of *exhaustion*, with cholesterol depletion once again. This rat was kept in the cold for 1 week.

All these changes in the gland might conceivably have been due to the rats being kept in the dark while in the refrigerator, rather than to the effect of the cold. To eliminate this possibility a further series of experiments was performed.

Series 4. Two rats were placed outside during the winter (1948) on cold bright days when the temperature registered was about 0° C. When one compares fig. 5, Pl. 2, showing the depletion of cholesterol from the cortex after 3 hr. of cold and dark, with fig. 6, Pl. 2, in which the depletion is even more extensive, from a rat exposed to cold and sunlight for 3 hr., one can conclude that the histochemical changes produced are not caused by the dark.

Attention may be drawn to certain points of interest. Cold without doubt causes varying degrees of cholesterol and lipoid depletion from the zona reticularis and zona fasciculata, but the zona glomerulosa appears to act independently and to remain non-depleted.

The Schultz positive sterols in the adrenal cortex are intracellular but extranuclear (cf. Yoffey & Baxter, 1949).

The fat around the gland never showed a positive Schultz reaction (see, for example, fig. 6, Pl. 2).

Discussion

The rapidity with which changes develop in the suprarenal cortex after exposure to cold is somewhat surprising. The earliest changes in the cortex described by Selye (1937) were noted 6 hr. after the administration of a variety of stimuli. Sayers, Sayers, Liang & Long (1946) described a fall in the ascorbic acid content of the whole gland, 1 hr. after the injection of pituitary adrenotropic hormone; they did not make any histochemical observations. However, Greep & Deane (1947) noted histochemically an alteration in the ascorbic acid content of rat suprarenals 6 hr. or more after administering desoxycorticosterone. Dosne & Dalton (1941), also working with rats, described a diminution of osmophilic material 1 hr. after exposure to cold (−3° C.). On the other hand, Flexner & Grollman (1939) observed an increase of osmophilic material in the suprarenal cortex of the rat half an hour after exposure to relatively moderate cold, 10° C.

In the present experiments, slight cholesterol depletion from the zona reticularis and fasciculata was observed as early as 5 min. after exposure to cold (fig. 2, Pl. 2) and even more markedly 15 min. after (fig. 3, Pl. 2). From the work of Sayers *et al.* (1946), Bloch (1945) and Yoffey & Baxter (1949) it is probable that the cholesterol diminishes because it is being utilized for the elaboration of steroid hormones. If this interpretation be correct, it would appear that steroid hormones can be formed and presumably discharged from the cortex far more rapidly than has generally been realized.

THE EFFECT OF ADRENALINE

Introduction

Little work has been reported on the extremely interesting effects of adrenaline on the adrenal cortex. Most outstanding is the work of Selye (1937), who demonstrated a typical alarm reaction on injecting 0·02 c.c. of 1/1000 adrenaline hydrochloride solution twice daily in a rat, and of Vogt (1944) who reported that in one decapitated dog an intravenous infusion of adrenaline caused a significant increase in the output of cortical hormone. Vogt (1945) further stated that the injection of adrenaline into rats three times a day for 8 days caused accumulation of cortical lipins as shown by Sudan III, and a still greater accumulation after 24 days of such injections, but that no such response to prolonged adrenaline treatment occurred in hypophysectomized rats.

Experimental details

The standard rat previously described was employed throughout.

0·036 c.c./100 g. body weight of 1/1000 adrenaline hydrochloride solution was injected intraperitoneally.

Immediately following even a single adrenaline injection all the rats behaved in a similar manner. They curled up and went to sleep. There was never any initial excitement or restlessness. The drowsiness persisted for 3–4 hr.

The same four histochemical tests were used as in studying the effects of cold, and the experiments fell into three series.

Series 1. To ascertain how soon after a single injection of adrenaline the adrenal cortex showed a histochemical change. Twenty-four rats were given a single injection

of adrenaline and killed at times varying from 5 min. to 3 hr. after this injection. It was found that 10 min. after such an injection there was a demonstrable cholesterol depletion particularly of the zona reticularis and inner part of the zona fasciculata, as shown in fig. 7, Pl. 2 and fig. 7, Pl. 1.

Series 2. How soon after a single injection of adrenaline does the gland return to normal?

Twenty-five rats were given a single injection of adrenaline and killed subsequently at varying time intervals up to 10 days. Three days after such an injection the histochemical picture had returned to normal (fig. 8, Pl. 1).

Series 3. Is it possible to demonstrate the stages of Selye's adaptation syndrome using adrenaline as the stimulus? The alarm stage is evident (fig. 7, Pls. 1 and 2) following a single injection of adrenaline.

Twenty-five rats were injected three times a day for periods ranging from 1 to 21 days; between the fourth and sixth days the gland changes from the alarm stage with its cholesterol depletion to the resistant stage with the re-accumulation of cholesterol (Pl. 1, figs. 9 and 10).

Fig. 11, Pl. 1 shows a gland, after 14 days of injections, passing into the stage of exhaustion, the cholesterol once more being depleted from the inner cortical zones, whilst fig. 12, Pl. 1, demonstrates complete absence of Schultz positive material from the cortex after 21 days of injection.

As with the investigation into the effects of cold it was found, making allowance for individual variation, that the general picture was the same with all the histochemical reactions used.

As far as the time factor is concerned, the adrenaline experiments fully confirm the observations already made in the case of cold, since cholesterol depletion has been noted as early as 10 min. after the intraperitoneal injection of adrenaline (fig. 7, Pl. 2).

Discussion

From the work of Selye (1937) it would appear that the effect of adrenaline on the suprarenal cortex is exerted through the pituitary. However, an experiment of Vogt (1944) pointed to the opposite conclusion, namely that adrenaline could cause an increased discharge of cortical hormone even in the absence of the pituitary. A direct action of adrenaline on the cortex would be an important part of the mechanism whereby the organism responds to cold, adrenaline being liberated by sympathetic stimulation. It might conceivably also have some bearing on the close association which has developed, in the course of vertebrate evolution, between cortex and medulla. This would further involve a special vascular relationship between cortex and medulla, to facilitate the passage of adrenaline from medulla to cortex. Studies of the vascular architecture of the suprarenal, made by the classical injection methods on dead material, cannot throw light on this. It may be significant in this connexion that the first indication of cortical depletion after exposure to cold is in the juxta-medullary region of the zona reticularis, but since a similar discharge from this region also occurs after the intraperitoneal or subcutaneous injection of pituitary adrenotropic hormone, acting after absorption into the blood stream, it would be unwise to attach much importance to this observation.

It is very interesting to note, however, that the juxta-medullary portion of the zona reticularis has an unusually rich blood supply (Gersh & Grollman, 1941).

SUMMARY

1. Cholesterol depletion from the zona reticularis and inner fasciculata of the adrenal cortex can be demonstrated histochemically after a 5 min. exposure to cold, and 10 min. after a single intraperitoneal injection of adrenaline hydrochloride.

2. The glands return to normal within 3 days after a 3 hr. exposure to 0° C., or after a single adrenaline injection.

3. The three stages of Selye's adaptation syndrome can be readily demonstrated by: (*a*) varying the length of time an animal is exposed to cold, and (*b*) giving repeated injections of adrenaline over long periods.

REFERENCES

Bloch, K. (1945). The biological conversion of cholesterol to pregnanediol. *J. biol. Chem.* **157**, 661–666.

Dosne, C. & Dalton, A. (1941). Changes in lipoid content of the adrenal gland of the rat under conditions of activity and rest. *Anat. Rec.* **80**, 211–217.

Elliott, R. T. (1914). Adrenal gland excision. *J. Physiol.* **39**, 38–53.

Flexner, L. & Grollman, A. (1939). The reduction of osmic acid as an indicator of adrenal cortical activity in the rat. *Anat. Rec.* **75**, 207–221.

Gersh, I. & Grollman, A. (1941). The vascular pattern of the adrenal gland of the mouse and rat and its physiological response to changes in glandular activity. *Contr. Embryol. Carneg. Instn*, **29**, 113–125.

Greep, R. & Deane, H. (1947). Cytochemical evidence for the cessation of hormone production in the zona glomerulosa of the rat's adrenal cortex after prolonged treatment with desoxycorticosterone acetate. *Endocrinology*, **40**, 417–426.

Hemphill, R. E. & Reiss, M. (1947). Regulation of endogenous cortin production. *Endocrinology*, **41**, 17–26.

Levin, L. (1945). Effects of several varieties of stress on the cholesterol content of the adrenal gland, and the serum of rats. *Endocrinology*, **37**, 34–43.

Sayers, G., Sayers, M., Liang, T. & Long, C. (1946). The effect of pituitary adrenotrophic hormone on the cholesterol and ascorbic acid content of the adrenal gland of the rat and guinea pig. *Endocrinology*, **38**, 1–9.

Selye, H. (1937). Studies on adaptation. *Endocrinology*, **21**, 169–188.

Vogt, M. (1944). Observations on some conditions affecting the rate of hormone output by the suprarenal cortex. *J. Physiol.* **103**, 317–332.

Vogt, M. (1945). The effects of chronic administration of adrenaline on the suprarenal cortex, and the comparison of this effect with that of hexoestrol. *J. Physiol.* **104**, 60–70.

Yoffey, J. M. & Baxter, J. S. (1949). Histochemical changes in the suprarenal gland of the adult male rat. *J. Anat., Lond.*, **83**, 89–98.

EXPLANATION OF PLATES

Plate 1

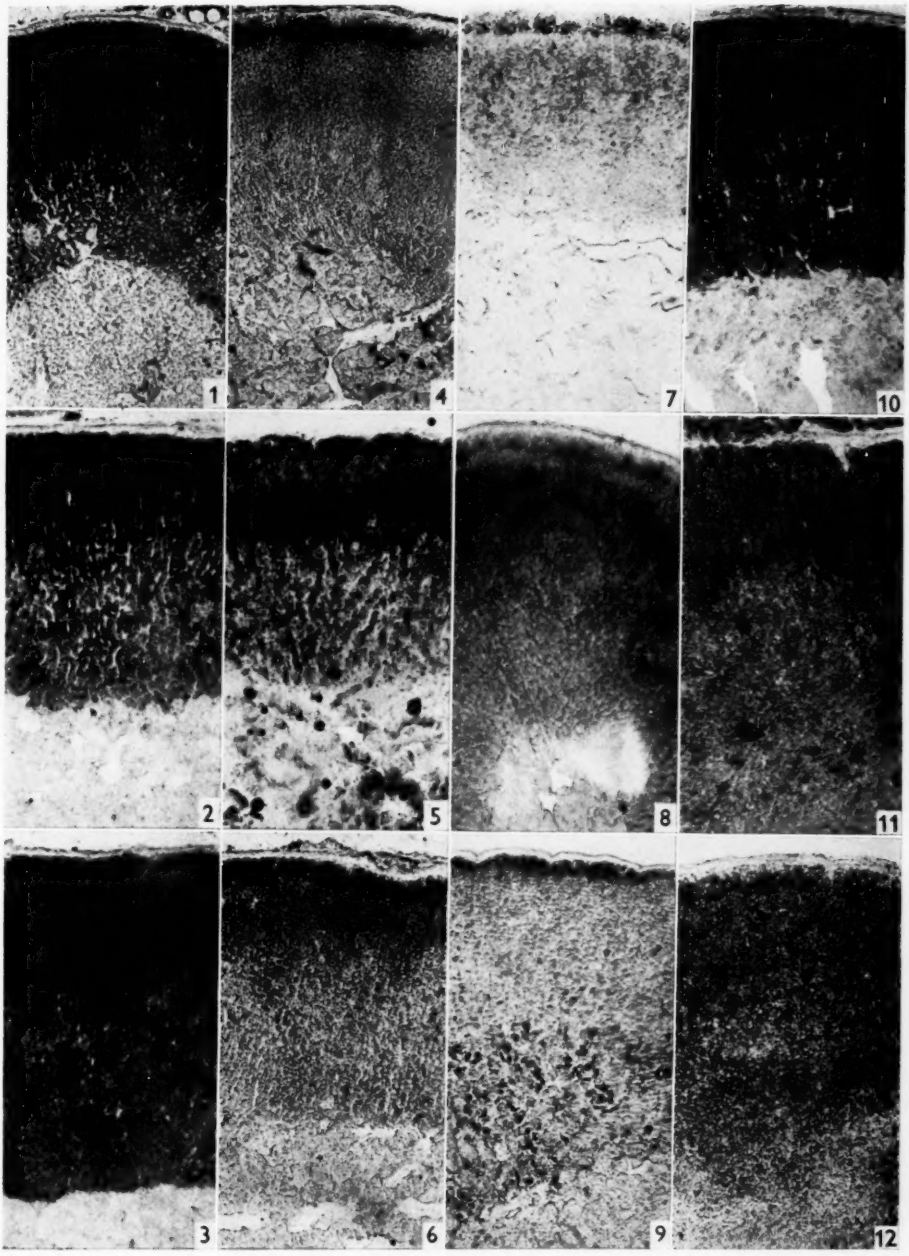
Figs. 1–3. The Schultz reaction in the adrenal cortex of 3 male adult rats, isolated for 7–10 days. Note Schultz positive material in all zones of cortex. ×50.

Fig. 4. Stage of alarm. Marked cholesterol depletion. Rat at 0° C. for 48 hr.

Fig. 5. Stage of resistance with a reaccumulation of cholesterol throughout the cortex. Rat at 0° C. for 72 hr.

Fig. 6. Stage of exhaustion—once more cholesterol is disappearing from the zona fasciculata and reticularis Rat at 0° C. for 1 week. ×50.

Figs. 7 and 8. Schultz reactions showing typical alarm depletion 10 min. after adrenaline injection (fig. 7), and a gland returning to normal 72 hr. after an injection (fig. 8). ×50.



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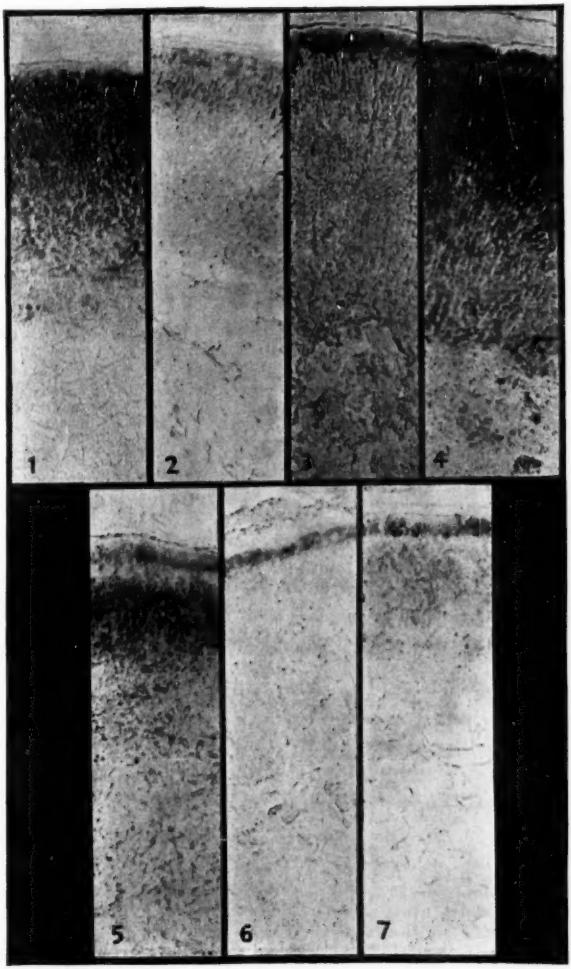

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Figs. 9 and 10. The change over from the alarm stage following adrenaline injection three times daily for 4 days (fig. 9), to the resistance stage after continued injections for 6 days (fig. 10). ×50.

Figs. 11 and 12. Both demonstrating glands passing into exhaustion. Fig. 11: cholesterol depletion in the inner zones of the cortex (compare fig. 10) following adrenaline injected for 14 days; and fig. 12: complete depletion of cholesterol from the cortex after 21 days of adrenaline injections. This black and white photograph is a little misleading. The zona fasciculata and glomerulosa were completely Schultz negative, but the brown of the section shows up darkly in the photograph. ×50.

PLATE 2 (COLOURED)

Fig. 1. Coloured photomicrographs of the Schultz reaction. A typical reaction in a standard rat. ×50.

Fig. 2. Depletion of Schultz positive material in the adrenal cortex of a rat kept at 0° C. for 5 min. ×50.

Fig. 3. Section showing still more pronounced cholesterol depletion in adrenal cortex of a rat at 0° C. for 15 min. ×50.

Fig. 4. An adrenal cortex filling up with cholesterol 72 hr. after a 3 hr. exposure to cold. ×50.

Figs. 5 and 6. Comparing these two sections, it can be seen that cold and light causes greater cholesterol depletion than cold and dark. Fig. 5. Cortex of a rat kept in the refrigerator for 3 hr. and killed 1 hr. later. Fig. 6. Cortex of a rat kept outside in cold and light at 0° C. for 3 hr. and then killed 1 hr. later. ×50.

Fig. 7 Typical alarm reaction. Rat given a single injection of adrenaline and killed 10 min. later. ×50.

GROWTH OF NERVE IMPLANTS IN VOLUNTARY MUSCLE

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INTRODUCTION

Attempts have been made in the past to produce an excess of nerve endings in a muscle (hyperneurotization) by implanting 'foreign' nerves, but the results have been contradictory. In most reports the findings were based almost entirely on the physiological tests of transmission between nerve and muscle rather than the subsequent histological examination of the implants, but Fort (1940), working with Weiss in Chicago, found that a foreign nerve implanted into a normally innervated muscle did not make either an anatomical or a functional connexion.

Gersuny (1906) is reported by Steindler (1916) to claim that functional endings were made by a foreign implant in a normally innervated muscle of dogs, and this was confirmed by Erlacher (1914) working on monkeys and guinea-pigs. Steindler (1916), working on dogs, and Elsberg (1917) on rabbits, both state that functional connexions were not made by implanted nerves in a normally innervated muscle. In all of this work it would appear that the operation of implantation and the methods used to retain the nerve in position (stitching) would cause considerable injury to the muscle. If muscle fibres were damaged or the normal nerve supply to a muscle fibre was cut, then a new end-plate would form on the denervated muscle fibre and on stimulation a small contraction would be expected.

Implantations of nerves into denervated muscles are reported by Steindler (1916), Elsberg (1917), Weiss (1930), Fort (1940), and others. Reinnervation of the muscles in varying degrees is claimed in all cases, but in the earlier works the histological appearances were not correlated with the observations made after the stimulation of the implanted nerve.

The results of the present investigation show that in a normally innervated muscle it is very difficult to produce extra motor end-plates on the muscle fibres, but that in a denervated muscle this hyperneurotization readily occurs. The problem of multiple innervation of muscle fibres has been much discussed in the past. If muscle fibres regularly and frequently have more than one end-plate, it might perhaps be expected that new motor end-plates would develop when a 'foreign' nerve is implanted into a normal muscle. This was not found in the present series of experiments.

MATERIAL AND METHOD

In all of these experiments rabbits were used. No special precautions were taken as to size and breed of animal. In most cases the sciatic and associated nerves were exposed by an incision through the biceps muscle. In some cases the nervus gastrocnemii medialis (n.g.m.) was cut near its muscle and its end implanted either into biceps or into the lateral head of gastrocnemius. In other cases the nervus peroneus was cut and implanted into the lateral head of gastrocnemius. Many of the nerves were implanted into portions of the muscle which are usually relatively free

of end-plates. The work of Couteaux (1942) was confirmed in that normal muscles, when stained, showed regions where no end-plates could be demonstrated.

It was found impossible to implant the nerve without some damage to muscle fibres, but this was kept to a minimum. A pathway was first made between the muscle bundles with a round-bodied needle for n.g.m., or a larger instrument for n. peroneus. On a number of occasions, use was made of a guarded crochet hook to pull the nerve into the muscle. This, however, produced a great deal of unnecessary damage, and the method was abandoned.

The distal end of the cut nerve was carefully inserted into the muscle wound and held in position with a drop of human fibrinogen which was coagulated with human thrombin. The use of fibrin removes the necessity of stitching the nerve into the muscle and so lessens the possibility of muscle damage. If abundant bleeding occurred during the implantation another site for implantation was prepared near to the original site. Two implants were usually made on each rabbit, on one side into normal muscle and on the other into a muscle whose nerve or tendon was cut at the same operation. The nerves to biceps and the lateral head of gastrocnemius were identified, thoroughly crushed and a length of 1·2 cm. excised. These precautions were probably adequate to prevent reinnervation of the muscles by their own nerves during the experiment.

After 100 days the animals were anaesthetized and the response of the muscles to stimulation of the nerves was obtained, using a faradic stimulator. Platinum wire electrodes embedded in Perspex were used and placed on the nerve 2–3 cm. from the muscle. The nerve was cut proximal to the electrodes. Care was taken to remove the excess fat and organized fibrin from the nerve, especially in the region of the electrodes. After observing the results of the stimulation, the nerve was crushed as it entered the muscle and again stimulated. The crushing of the nerve blocked all indirect excitation of the muscle but did not prevent direct excitation due to surface spread or 'escape' of the stimulus.

After stimulation, the muscle with nerves attached was dissected out and placed in fixative. The type of fixation was varied according to the method of staining. Trichloracetic acid (5 %) was found to be a good fixative for a future Bodian protargol staining, but results were not consistent. After Bodian fixation (80 c.c. of 80 % alcohol, 15 c.c. of commercial formalin and 5 c.c. of glacial acetic acid), and celloidin embedding some specimens were stained with Holmes's modified Cajal technique, using buffered solutions. The most successful method was found to be fixation in 10 % formalin with 2 % pyridine in water for at least 7 days, the muscle being then washed, and frozen sections made at about 50 μ. These were stained by a modified Bielschowski-Gros technique, using pyridine as a buffer in the reducing solution of formalin. The method was as follows:

(1) Impregnation for 20 min. in the dark in 10 % aqueous solution of silver nitrate.

(2) The sections are removed and passed directly into an aqueous solution of 10 % formalin (acid) containing 2 % pyridine. This is the first of four washings in this formal-pyridine solution and the times are roughly 30 sec., 1, 4 and 8 min.

(3) After removing excess formal-pyridine the sections are placed in ammoniacal silver solution for 1 min. This solution is made by adding 880 ammonia drop by drop

to 5 c.c. of 10 % $AgNO_3$ solution until the precipitate has disappeared. Ten to fifteen further drops were then added. The importance of adding the ammonia in small quantities was shown by Silver (1942), and was confirmed in present experiments. The amount of ammonia added will vary with the pipette or dropper, but must be controlled by the staining of the section. The section should be a very faint brown colour. If too much ammonia is added, the staining is inhibited and if too little, the sections are dark and the connective tissue and vascular elements stain. The sections should be gently agitated during the time in ammoniacal silver solution.

(4) Transfer to water containing few drops of 880 ammonia for about 10 sec.

(5) Transfer to water containing few drops of glacial acetic acid for about 10 sec.

(6) Wash in water. The sections should be almost colourless. If they are brown then the ammoniacal silver has contained too little ammonia—probably due to evaporation. This is specially seen in hot weather.

Usually the sections were toned in 1 % gold chloride until a faint deposit could be seen on them (about 10–30 sec.). They were then fixed in a 5 % hypo solution and were then washed, dehydrated, cleared in creosote, and mounted in Canada balsam. It should be remembered that in both the toning solution and the fixing solution, bleaching occurs and loss of the finer endings will be noted if the sections are allowed to stay too long in these solutions.

To facilitate identification later, it was found advisable to mark the site of implantation and one corner of the block of muscle with indian ink. This did not interfere with the staining but was valuable as the sections could be mounted symmetrically.

The thick sections which can be stained and examined after staining by the Bielschowski-Gros methods have many advantages, though the difficulty of obtaining serial sections is a marked disadvantage.

RESULTS

Electrical stimulation of the implanted nerves gave rise to responses which were usually rather weak. When contraction of the muscle was obtained it was seen to be occurring in those fibres in the vicinity of the implant and there was never a total response of the whole muscle unless the stimulus was so strong that the effect was probably due to surface spread of the stimulus. When contraction occurred, then those fibres which responded to the stimulus did so, as far as could be detected, in their total length. Almost all the denervated muscles showed fibrillation.

In Table 1, there is listed against each animal an estimate of the extent of the muscle response to stimulation of the implanted nerve. In no experiment was the contraction vigorous enough to move the leg or the foot. Thus we have twenty cases of nerves successfully implanted into denervated muscles, and seventeen of these showed clear signs of indirect excitability through the nerve, as estimated by response at the threshold of 25 cm. or more coil distance, controlled by crushing of the nerve peripheral to the point of stimulation. In three cases the threshold was such as to raise suspicion that the response was due to 'escape' of current to the muscle fibres.

Out of seventeen cases in which implants were made into muscles with a normal nerve supply, nine showed response on stimulation of the nerve with currents weak

enough to be regarded as providing unambiguous evidence of indirect excitability. The remaining eight showed 'response' only to very strong shocks. In these cases, the response was probably due to escape of the current. The indirect responses of the muscles with the normal nerve supply were much weaker than the responses of the muscles which had previously been denervated. The results shown in Table 1 show that the implant makes a functional connexion more frequently and easily in a denervated muscle than in a normal muscle. This agrees with the previous finding (Aitken, 1949) that when a nerve regenerates into a denervated muscle its fibres mature further than do fibres growing into a muscle already innervated. The more mature nerves had many fibres whose total diameter (axon and myelin sheath) was more than 6μ and few fibres under 6μ. The less mature nerves had large numbers of small fibres (under 6μ) and few large fibres.

Table 1. *Against each animal there is given an estimate of the extent of the response to indirect excitation of the muscle by the implanted nerve*

N.g.m. = nervus gastrocnemii medialis; ○ = nerve pulled out; − = no response; + = slight response; + + = vigorous response.

Animal	Nerve	Response on 'normal' side to indirect excitation	Response on 'paralysed' side to indirect excitation
398	N.g.m.	−	+
404	N.g.m.	+ +	+
417	N.g.m.	+	+ +
418	N.g.m.	−	−
428	N.g.m.	+	+
443	N.g.m.	−	+
444	N.g.m.	+	+ +
446	N.g.m.	−	○
458	N.g.m.	○	+
459	N.g.m.	○	+ +
483	N.g.m.	−	+ +
484	N.g.m.	+	+ +
642	N.g.m.	+	○
643	N.g.m.	+	−
395	N. peroneus	○	+ +
446	N. peroneus	−	+
458	N. peroneus	○	−
459	N. peroneus	+	+
483	N. peroneus	+	+
540	N. peroneus	○	+
880 (50 days)	N. peroneus	−	+ +
881 (50 days)	N. peroneus	−	+ +

EXAMINATION OF THE IMPLANTS IN MUSCLE

A. *Implants in normally innervated muscle*

The regenerating nerve fibres grow out from the cut end of the nerve and form a tangled mass of fine fibres (Pl. 1, figs. 1, 2). Generally speaking the fibres are thin and lightly myelinated or non-myelinated. They pass between the muscle fibres and no very obvious cellular reaction is observed either in the connective tissue or the muscle. The fibres can often be followed for long distances and seem to end in the endomysium. The Schwann cell nuclei (Pl. 1, fig. 3) vary in their staining intensity. Some are intensely black, as though the silver were deposited on the surface, whilst in others the nucleolus and other nuclear structure can be seen. Other nuclei are mostly unstained and appear as mere shadows. Some of the nuclei probably belong to fibroblasts. In

a suitable preparation the connective tissue network can be stained and is quite different from the nerves. The undulations of the fibrous tissue are smaller and more frequent and the cell nuclei are less frequently seen (Pl. 1, fig. 4).

In those specimens in which an unmyelinated fibre lay in close proximity to a muscle fibre (sometimes for a considerable distance), no change in the constitution of the muscle was detected. Special attention was paid to the muscle nuclei, but they were neither more conspicuous nor altered in their distribution. There was no accumulation of sarcoplasm as is found in a normal motor end-plate.

If the implanted nerve grows into a region of the muscle which has motor end-plates (Pl. 1, fig. 5), then the regenerating fibres pass near to the end-plates, but there was no evidence of either attraction or repulsion between them.

These fibres, although they make no visible endings, frequently develop a myelin sheath (Pl. 1, fig. 6). This was also seen in the cross-sections of the regenerating nerves which were stained by the Flemming-Weigert method (Aitken, 1949). A few fibres reach 10–12μ in diameter (axon and myelin) but most of them are about half this size.

Occasionally, one or two atypical motor end-plates are found near to the neuroma. These are probably formed on a portion of a muscle fibre which has been damaged during the implantation and severed from the part of the fibre that carries a normal motor end-plate. It is interesting to speculate on the conditions in which a detached portion would call forth a response from the nerves. Pl. 1, fig. 7 shows one of these atypical motor end-plates. Though this muscle was normally innervated, the tendon had been cut at the operation. Native endings were found in abundance, and in addition a few of these new motor end-plates. The new end-plates can be identified by their position (near the neuroma) and by the thin fibre with little or no myelin which leads to them. These damaged muscle fibres, which had received a new motor ending, would contract on stimulation and would be the explanation of the contractions found in some specimens. These contractions were restricted to the vicinity of the implant and did not spread through the muscle.

Contractions of muscle were found in some specimens in which no motor end-plates at all could be demonstrated. Though serial sections were not made of the muscles, care was taken that very little tissue was lost and all sections were examined. If these cases have been correctly interpreted it must be supposed that the fine fibres in close proximity to the muscle fibres were able to stimulate the muscle fibre.

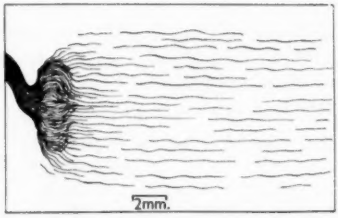
B. *Implants in denervated muscle*

(1) In a denervated muscle, the histological picture of the neuroma and regenerating fibres is very different. Examination with the low power of the microscope (Pl. 1, fig. 8) shows the regenerating fibres branching in the muscle. The fibres branch in an irregular manner and end-plates are formed soon after the division. The pattern of innervation is very like that found in an intact muscle where the large nerve fibres are seen to run across the muscle fibres and only the smaller fibres run parallel with the muscle fibres. The pattern of the nerves is quite different from that made by nerves which have been implanted into a normal muscle (Text-fig. 1).

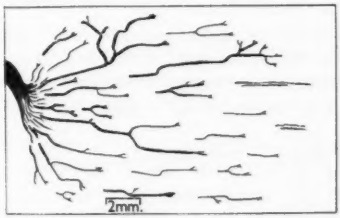
Most of the nerves are myelinated and as seen in Pl. 1, fig. 9, the thick myelin sheath is retained to within a few microns of the end-plates. In Pl. 2, fig. 10, one

of these myelinated nerves is seen dividing into three branches. Occasionally a large myelinated fibre is seen to travel for a considerable distance. In Pl. 2, fig. 11 the fibre travels about 0·8 mm. before it divides into the smaller branches which give rise to the motor end-plates. Beyond the region where motor end-plates have formed, but in the same plane of section, the nerve fibres travel for long distances between the muscle fibres. These muscle fibres presumably have already been reinnervated and the surplus nerve fibres from the growing end of the implant pass between them. The picture in Pl. 2, fig. 12, which is an adjacent field to that in Pl. 1, fig. 8, is here very like that obtained when an implant is made into a normal muscle.

(2) Motor end-plate production. The great majority of the newly formed 'foreign' motor end-plates differ greatly in size and shape from the normal end-plate which is found in a normal muscle, but some are very like normal endings. Pl. 2, fig. 13 shows one nearly normal ending. The entering fibre divides and the two branches appear to enter opposite poles (ends) of the plate. The nucleus of a Schwann cell is seen on one branch and the end-plate nuclei are clearly seen.



Drawing of nerve implant in normal muscle



Drawing of nerve implant in denervated muscle

Text-fig. 1. Drawings of two nerve implants. The nerves in the normal muscle travel for long distances with little sign of branching. The nerves in the denervated muscle branch very frequently and irregularly.

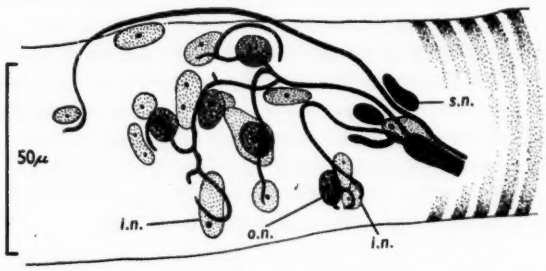
Some of the end-plates are supplied from two nerve fibres as can be seen in Pl. 2, fig. 15. Is this an example of the reinnervation of an empty normal end-plate? In this series of experiments, no evidence was seen of these denervated end-plates which, according to Gutmann & Young (1944), usually show as more darkly staining areas.

The more atypical plates varied from large claw-like endings on a muscle fibre to groups of small endings on a muscle fibre. In Pl. 2, fig. 16, one of the large claw-like endings is seen. Text-fig. 2 is a drawing of this ending. There are two main branches to this end-plate and at least nine terminal branches. The number of inner end-plate nuclei is eleven, and of outer end-plate nuclei, five. Three Schwann cell nuclei can be made out. The main nerve trunk is myelinated almost as far as the end-plate, whose overall size is 0·075 by 0·05 mm. All of these figures are within the range of figures given by Gutmann & Young (1944) for normal end-plates and reinnervated end-plates. The width of the muscle fibre at the region of the end-plate was 0·09 mm.

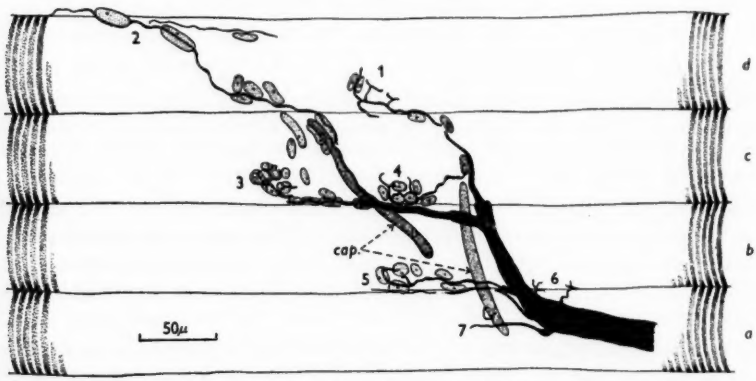
A third fine fibre can be traced past the end-plate to finish on the surface of the muscle fibre some distance beyond.

Occasionally there is found a large axon which divides to supply motor end-plates to a group of muscle fibres. In Text-fig. 3, a drawing of one of these large nerve trunks, there are several muscle fibres each with a well-formed and a very irregular

ending. Owing to slight differences in level and to the thickness of the section, it is impossible on a single photograph to show the detail, but Pl. 2, fig. 17 is a photograph of this group of endings. A muscle fibre '*a*' has one plate ending, and '*b*' has two and possibly a third, '*c*' has two and '*d*' has one end-plate and one fine fibre which comes in contact with the muscle fibre and appears to produce changes in the orientation of the muscle nuclei.



Text-fig. 2. Drawing of large new motor end-plate in muscle fibre. Note the number of Schwann cell nuclei and end-plate nuclei. A small nerve fibre which accompanies the large fibre passes by the end-plate and forms a small bare ending on the muscle surface. Abbreviations: *i.n.*, inner end-plate nucleus; *o.n.*, outer end-plate nucleus; *s.n.*, Schwann nucleus.



Text-fig. 3. Drawing of a group of large irregular motor endings which are derived from one large nerve trunk. Four muscle fibres (*a*, *b*, *c*, *d*) are each innervated by two or more end-plates (1, 2, 3...). *cap.*, capillary.

In the experiments where the nerves regenerate into a paralysed muscle two or more motor end-plates are frequently found on one muscle fibre. Pl. 3, fig. 18 shows an example of this. At least four end-plates are present and appear to be on one muscle fibre. Careful focusing shows that they are all at about the same level in the muscle. Even if they are on contiguous surfaces of two muscle fibres there is still good evidence for multiple innervation of the muscle fibres. These endings, however, all appear to arise from the same axon and so the innervation is uni-axonal and therefore unisegmental. Pl. 2, fig. 15 shows an ending which is bi-axonally innervated.

Examination of the denervated muscles showed that the amount of interstitial fibrous tissue had increased and the fat cells were very conspicuous. The differences between the normal and paralysed muscles were obvious from the beginning of the histological process. The paralysed muscle cut much more easily and the sections tended to float at the top of the collecting tubes. The blood capillary plexus always stained well in the paralysed muscle unless special care was taken to suppress it.

C. *Implants into tenotomized muscles*

The normal motor end-plates after tenotomy do not appear to be very different from those seen in a normal muscle (Pl. 3, fig. 19). Some implants (Pl. 3, fig. 20) give rise to many long thin fibres which coil up between and around the muscle fibres. This is not seen in nerves which are growing into normal muscle. Sometimes one of the coiling fibres (Pl. 3, fig. 21) is seen to form a large loop, the terminal branch turning back on the main fibre. Smaller side branches leave the same nerve. Many of the fibres, as in implants in normally innervated muscles, are myelinated (Pl. 3, fig. 22) and Schwann cell nuclei and nodes of Ranvier can be clearly seen. On some fibres the neurilemma can also be made out.

Other implants give rise to fibres which stream away between the muscle fibres (Pl. 3, figs. 23, 24). They travel for long distances and then taper away. No muscle cell or interstitial cell reaction was observed. Myelin sheaths are very prominent (Pl. 3, fig. 24) with nodes of Ranvier and Schwann cell nuclei.

Careful inspection of the material showed no regenerating nerves which had undoubtedly formed new motor end-plates. Such plates as are present are so typical in their characteristics that they are probably the normal endings.

DISCUSSION

The purpose of this investigation was to find an answer to the question: 'Will new motor end-plates develop in a muscle as the result of the entrance of 'foreign' nerve fibres?' It has been shown that in a denervated muscle the 'foreign' nerve readily produces new end-plates, which function when the nerve is stimulated. In a normal muscle, the 'foreign' nerve usually produces many long thin nerve fibres, which ramify amongst the muscle fibres and rarely form a muscle end-plate. In some experiments, however, the normal muscle contracted in response to stimulation of the implanted nerve; it is therefore probable that some of these nerve fibres make a functional connexion with the muscle fibres. Reinnervation is certainly very much easier and more abundant in muscles which had previously been denervated.

The observations made during these experiments raise two further questions. (1) What is it that prevents the regenerating fibres from forming new motor end-plates in normal muscle? (2) Would it be possible by implantation to reinnervate a muscle which had been paralysed (by denervation)?

(1) This problem has received much attention from workers who are interested in the innervation of muscle. Multiple endings, pluri-axonal and pluri-segmental, have been reported in Amphibia and Reptilia and there is good evidence—both histological and physiological—to support this view (Kühne, 1887; Katz & Kuffler, 1941), though other workers (Kulchitsky, 1924; Wilkinson, 1929) found only one ending. In mammals, however, the evidence is not so clear. Multiple motor endings on the

intrafusal muscle fibres of muscle spindles have been demonstrated by Cuajunca (1932), Barker (1948), and others. On the extrafusal muscle fibres, Agdhur (1916) illustrates two endings, one above the muscle fibre and the other on the deep surface. Wilkinson (1929) made a very full and careful survey of many types of animal and inspected the preparations of Agdhur and others. He was of the opinion that as a rule one ending is found on a muscle fibre and that if more than one occurs it must be a great rarity. Harrison (1910) and Tello (1917) have suggested the analogy of the ovum and sperm as a fair comparison with muscle fibre and end-plate. It may well be, however, that there is a relationship between the length of the muscle fibre and the number of end-plates on it. In the experiments which have been described it would appear that the normal muscle was already in a state of nerve-muscle equilibrium and that no further end-plates would form on the muscle fibres. Occasionally, however, the implanted ('foreign') nerve produced motor end-plates. In these cases it may be that the normal nerve to the muscle fibre had been cut or, more probably, the muscle fibre had been divided into a part with the normal end-plate and a part without an end-plate. In either case the 'foreign' nerve would presumably be able to form a new end-plate. If the detached portion of the muscle fibre is reinnervated, then whatever the cause of the non-receptiveness, it is a reversible phenomenon and depends on the close association with the end-plates.

If there is a quantitative relationship between the length of the fibre and the number of end-plates it should be possible to devise experiments in which the muscle fibres are broken into varying lengths. End-plates might perhaps be induced to form more readily on the longer portions than on the shorter.

Most of the end-plates in a muscle are arranged in a more or less regular pattern. When the muscle fibres are parallel, as in sartorius, the endings are found in a band across the muscle. Some parts of the muscle, such as the upper end of sartorius, are free of endings. In gastrocnemius and biceps femoris it is possible to make the implantation into a region which is devoid of normal motor end-plates. When this was done, new end-plates readily developed in a denervated muscle. If the implants were in the region of the normal end-plates, then some of these may have been reinnervated. If reinnervation had occurred regularly then the new end-plates formed by the 'foreign' nerve should follow the pattern of the normal end-plates shown by Couteaux. The end-plates were, however, found scattered through the muscle, so no evidence was found for a neuropathic attraction of end-plates on the regenerating nerves.

From the appearances of the regenerating nerves in the denervated muscle, it is evident that the muscle fibres had a marked effect on the nerves. After the nerves came into contact with the denervated muscle fibres the nerves divided up in a complicated manner, and it appears that as soon as a small branch came into very close contact with the muscle fibre, the nerve divided into numerous fine branches forming an end-plate. This is well seen in Pl. 2, fig. 11, which shows a nerve fibre crossing over numerous muscle fibres without any sign of division, and then suddenly giving rise to short branches which finish as motor end-plates on muscle fibres.

In the denervated muscles, an implanted nerve produced motor end-plates on the muscle fibres at all positions along the muscle. There did not appear to be any area which was more easily innervated than another. It would therefore appear that all

points of a denervated muscle fibre are equally receptive and capable of forming an end-plate. What is more, in these reinnervation experiments, more than one end-plate is frequently found on the muscle fibre. Sometimes the two end-plates are 'in parallel', the two fibres which supply them being about equal length and size. These end-plates probably developed on the muscle fibre about the same time (see Pl. 3, fig. 18). In other cases, the end-plates are 'in series' (Pl. 3, fig. 17 and Text-fig. 3), and there must have been a short interval of time between the formation of the two plates. The muscle fibre was unable, in the time available, to become non-receptive to other fibres. Pl. 2, fig. 11 shows, however, that within a short period of time the nerves can meet with non-receptive muscle fibres, and Pl. 2, fig. 12 shows the long straggling fibres which are found near to the new end-plates. These fibres are presumably travelling between muscle fibres which have already been reinnervated. The implants in normal muscle show this non-receptive state in a marked degree.

(2) These experiments showed that there can be reinnervation of a denervated muscle by an implanted nerve. The new end-plates, however, were found in the region of the end of the implant and not over a wide area of the muscle. This may be due to the short duration of the regenerative period. Certainly the implantation of a number of small nerves or the fascicles of a larger nerve would have a better chance of reinnervating the whole paralysed muscle.

The results which have been obtained show that the great excess of regenerating nerve fibres which is produced in a normal muscle when a 'foreign' nerve is implanted in it does not produce hyperneurotization of the muscle.

SUMMARY

1. A method of implanting nerves in muscle with the minimum of injury to the muscle is described.

2. Nerves which regenerated into a denervated muscle formed functioning motor end-plates.

3. Nerves which regenerated into normal muscle rarely formed motor end-plates and these were probably on muscle fibres that had been damaged at operation.

4. In the implants in denervated muscles, more than one ending was frequently seen on a muscle fibre.

5. Implants into tenotomized muscles formed long thin fibres which travelled between the muscle fibres and occasionally coiled round them. No regenerated endings were seen.

6. The non-receptiveness of normal muscle fibres is discussed.

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REFERENCES

AGDHUR, E. (1916). Morphologischer Beweis der doppelten (plurisegmentalen) motorischen Innervation der einzelen quergestreiften Muscelfascun bei den Säugetieren. *Anat. Anz.* **49**, 1–13.

AITKEN, J. T. (1949). The effect of peripheral connexions on the maturation of regenerating nerve fibres. *J. Anat., Lond.*, **83**, 32–43.

BARKER, J. (1948). The innervation of the muscle spindle. *Quart. J. micr. Sci.* **89**, 143–86.

COUTEAUX, R. (1942). La cholinesterase des plaques motrices après section du nerf moteur. *Bull. biol.* **76** 14–57.

CUAJUNCA, F. (1932). The innervation of the neuromuscular spindles. *J. Comp. Neurol.* **54**, 204–26.

ELSBERG, C. A. (1917). Experiments on motor nerve regeneration and the direct neurotisation of paralysed muscles by their own and foreign nerves. *Science*, **45**, 318–20.

ERLACHER, P. (1914). Ueber die Motorischen Nervenendigungen. *Z. orthop. Chir.* **34**, 561–85.

FORT, W. B. (1940). An experimental study of the factors involved in the establishment of neuromuscular connections. University of Chicago Libraries, Illinois.

GERSUNY, A. (1906). Eine Operation bei motorischen Lähmungen. *Wien. klin. Wschr.* 10.

GUTMANN, E. & YOUNG, J. Z. (1944). The reinnervation of muscle after various periods of atrophy. *J. Anat., Lond.*, **78**, 17–43.

HARRISON, R. G. (1910). The outgrowth of the nerve fibre as a mode of protoplasmic movement. *J. exp. Zool.* **9**, 787–848.

KATZ, B. & KUFFLER, S. W. (1941). Multiple motor innervation of the frog's sartorius muscle. *J. Neurophysiol.* **4**, 209–23.

KÜHNE, B. (1887). Neue Untersuchungen über motorische Nervenendigungen. *Z. Biol.* **23**, 1–148.

KULCHITSKY, N. (1924). Nerve endings in the muscles of frogs. *J. Anat., Lond.*, **59**, 1–19.

SILVER (1942). Colloidal factors controlling silver staining. *Anat. Rec.* **82** (Suppl.), 507–29.

STEINDLER, A. (1916). Direct neurotisation of paralysed muscles. Further studies of the question of direct nerve implantation. *Amer. J. orthop. Surg.* **14**, 707–19.

TELLO, J. F. (1917). Genesis de las terminaciones nerviosas motrices y sensitival. I. En el sistema locomotor de las vertebra-superiores. Histogenesis muscular. *Trab. Lab. Invest. biol. Univ. Madr.* **15**, 101–99.

WEISS, P. (1930). Neue experimentelle Beweise für das Resonanzprinzip der Nerventätigheit. *Biol. Zbl.* **50**, 357–72.

WILKINSON, H. J. (1929). The innervation of striated muscle. *Med. J. Aust.* **2**, 768–93.

EXPLANATION OF PLATES

All sections were stained by a modified Bielschowski-Gros technique.

Figs. 1–7. Implants in normally innervated muscles.

Figs. 8–18. Implants in denervated muscles.

Figs. 19–24. Implants in tenotomized muscles.

PLATE 1

Fig. 1. N. peroneus implanted into normal lateral head of gastrocnemius shows long, thin, unbranched fibres. (483 B 9.)

Fig. 2. Same section under higher power of microscope.

Fig. 3. Implanted nerve fibre in normal muscle showing different staining of Schwann cell nuclei. (484 D 6*a*.)

Fig. 4. Section of paralysed biceps showing muscle reticulum. Note the small number of nuclei as compared with that found on a nerve fibre. Compare with fig. 3. (881 H 1.)

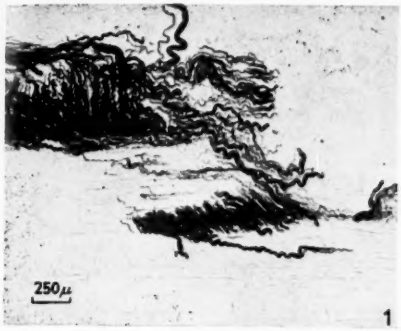
Fig. 5. N.g.m. implant near native endings. (856 G 14.)

Fig. 6. N.g.m. implant in normal muscle showing presence of myelin sheath on fibres. (484 D 14.)

Fig. 7. N. peroneus implanted into tenotomized gastrocnemius and showing large newly formed end-plates due to injury to muscle fibres during implantation. (251 B 2.)

Fig. 8. Pattern of reinnervation of paralysed gastrocnemius by implanted n. peroneus. Note the thick nerve fibres which frequently branch. (395 A 13.)

Fig. 9. Large myelinated fibres from an implant in paralysed muscle. The end-plates form at the side of the main trunk. Though only three end-plates are in view, focusing in different levels brought up three other smaller end-plates. (855 E 6.)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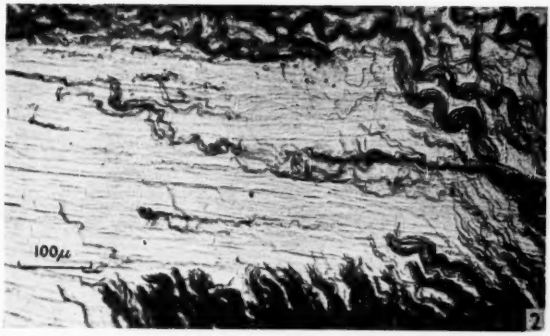
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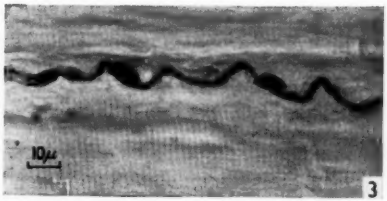
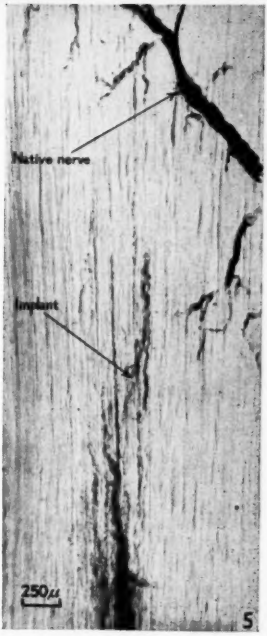
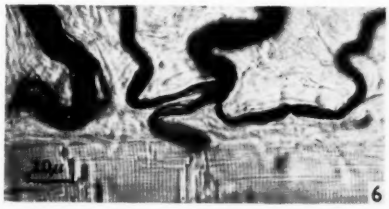
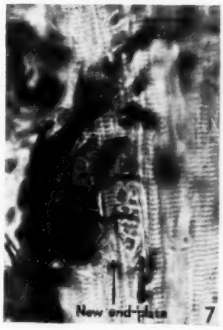
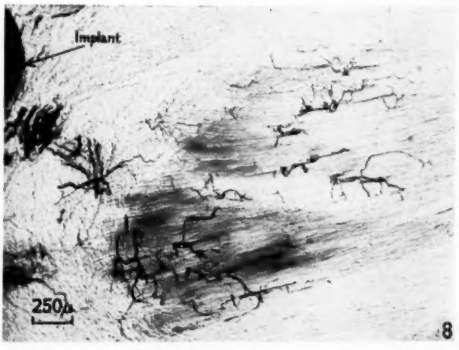

10μ
3

50μ
4


Native nerve
Implant
250μ
5

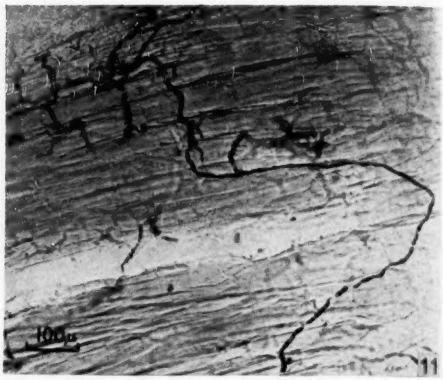

10μ
6


New end-plate
7


Implant
250μ
8

100μ
9

100μ
10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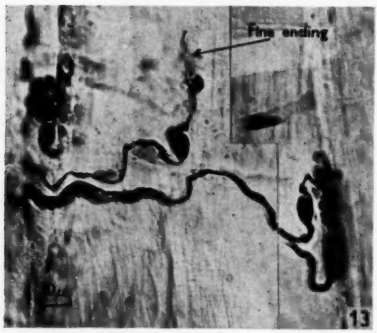
Fine ending
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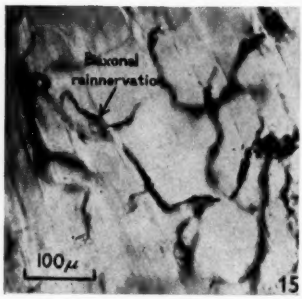
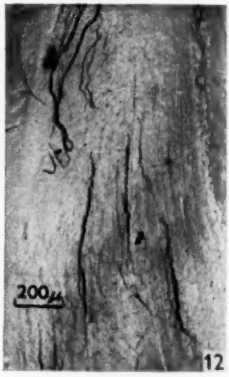
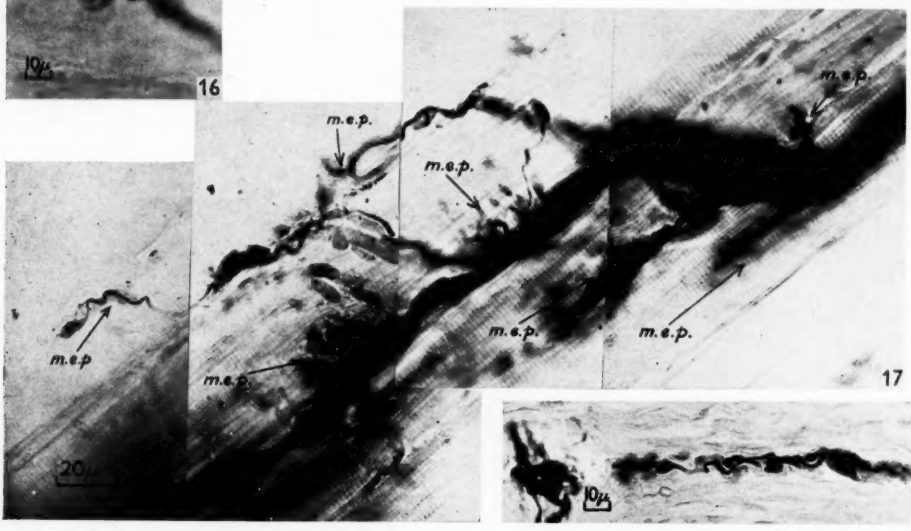

Paraxonal
rainnervation
100μ
15


200μ
12

10μ
16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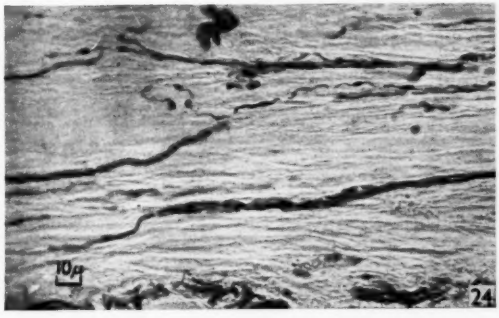
10μ
24

PLATE 2

Fig. 10. Showing the division of myelinated fibre into three branches. (395 A 13.)
Fig. 11. Myelinated nerve from the implant which travels a considerable distance before dividing into smaller branches with terminal end-plates. (395 A 16.)
Fig. 12. Implant into paralysed muscle. Long thin straggling fibres passing between muscle fibres which have already been reinnervated. (395 A 13.)
Fig. 13. New motor end-plates formed in paralysed muscle by the implanted nerve. Note the Schwann cell nuclei, end-plate nuclei and the fine ending of the upper fibre. Retouched. (484 B 6*b*.)
Fig. 14. Fine nerve fibre lying in close contact with muscle fibres. Some Schwann cell nuclei can be seen but many of the others present probably belong to the muscle. (484 B 6*a*.)
Fig. 15. Bi-axonal innervation of a new end-plate formed in paralysed muscle. (855 E 3.)
Fig. 16. Large new motor end-plate formed in paralysed muscle. Compare with Text-fig. 1. (855 E 3.)
Fig. 17. Group of large new endings on paralysed muscle. Compare with Text-fig. 2. (855 E 4.) Motor end-plates labelled *m.e.p.*

PLATE 3

Fig. 18. Group of four new endings on side of muscle fibre. All the endings are derived from the one large nerve trunk. (395 A 13.)
Fig. 19. Native motor end-plates in a tenotomized gastrocnemius. (251 B 39.)
Fig. 20. Implant of n. peroneus into tenotomized gastrocnemius. Note the long thin straggling fibres. A group of native endings is present at the left side of the picture. (251 B 39.)
Fig. 21. Example of a long thin straggling nerve winding between muscle fibres and forming almost a complete circle. (251 B 39.)
Fig. 22. Myelinated fibres of n. peroneus which was implanted into tenotomized gastrocnemius (251 B 19.)
Fig. 23. N.g.m. implanted in tenotomized gastrocnemius. Note long thin nerves passing between muscle fibres with no sign of branching. (251 D 36.)
Fig. 24. High-power view of these unbranching fibres. Note myelination. (251 B 36.)

A NOTE ON THE SPECIFICITY OF THE CORNEAL EPITHELIUM

By R. E. BILLINGHAM and P. B. MEDAWAR
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It is a well-known fact that much of the finer detailed differentiation of the adult body is brought about by the direct adaptive responses of tissues to their environment. The architecture of a bone may be remodelled under a changed system of stresses; muscles enlarge upon constant use; skin thickens where it is irritated, darkens upon exposure to sunlight, and creases where it is repeatedly folded. With such evidence in mind it would be natural to suppose that the detailed histological differentiation that represents the final stage of embryonic development was also in large part governed by differences of environment and of habit of use. For example, the thick and heavily keratinized epidermis of the sole of the foot, like that of a corn or callosity, differs from the relatively quiescent skin of the body surface by its intense and sustained mitotic activity. This might very well be the consequence of the constant pressure and irritation to which the epithelium of the sole has to submit—the more obviously so, since callosities can be produced by such means at will. It has, nevertheless, been shown experimentally (Billingham & Medawar, 1948*a*) that the difference between sole and body epithelium, though primarily functional and not anatomical, is innate and inherited. A corn or callosity does not survive indefinitely the irritation that provoked its formation; but sole skin grafted from the foot of the guinea pig to its chest, where it is secure from irritation and protected by the overgrowth of hair from the skin around it, perseveres in its intense mitotic activity and continues to manufacture layer after layer of thick and now functionless cuticle throughout the life of the animal—in our experience, for more than two years after operation. The fact that there exist *inherited* differences between somatic cells of a type that can be and very often are mimicked by direct adaptive responses to the environment is of some importance in evolutionary speculation, since it offers special temptations to the Lamarckist. (The difference between 'inborn' and acquired flexure lines is in principle of this same sort: acquired flexure lines may disappear, but the plastic surgeon affirms that skin carrying 'inborn' flexure lines retains them wherever it may be transplanted.)

The epithelium of adult guinea-pig's tongue, claw, and ear skin (Billingham & Medawar, 1948*a*, and unpublished) have each likewise been shown to retain their exact specificity of histological type upon heterotopic transplantation. In this paper it will be shown that the same is true of the corneal epithelium, and that the maintenance of its peculiar properties does not depend upon its continuing to live in its physiologically unique environment. The epithelium of the cornea differs from that of body skin in its perfect transparency and in its mode of cell packing, which is peculiarly compact at all levels, no flaking cuticle being formed. Its mode of attachment to the stroma is not known, but it is known not to depend upon the presence of elastic fibres. Corneal epithelium is indeed peculiarly mobile upon its substratum, as

studies on corneal healing have repeatedly shown. So far as can be judged by mere microscopic observation, the corneal epithelium consists of cells of one lineage only, and the cells correspond with the 'ordinary Malpighian cells' of body epidermis. We have not been able to reveal an epidermal glial system of dendritic cells in the corneal epithelium (cf. also Redslob, 1922) such as is found within the basal layer of guinea-pig and human body skin epithelium (Billingham, 1948, 1949), though they are present in the conjunctival epithelium at the corneal limbus. (Peculiar branched wiry bodies may be identified immediately below the basal layer of corneal epithelium by their intense supravital staining with methylene blue (Pl. 1, figs. 5–7), but there is no evidence that these are dendritic cells or, indeed, that they are cells at all. They are not to be confused with the well-known branched connective tissue cells of the corneal stroma.) It is not known that the corneal epithelium contains any cells of the lineages which, in body skin, must be presumed responsible for formation of sweat and sebaceous glands and other epidermal appendages. Unfortunately, our knowledge of the cell lineages that comprise the epithelium of body skin is at present quite rudimentary, and no more can be said about them here.

The maintenance of the peculiar transparency and cell packing of the corneal epithelium might well depend in some degree on the peculiar physiological properties of its environment. Being moist, the corneal epithelium during waking hours is probably the coolest tissue in the body, and lacking a blood supply it must live at a much lower oxygen-tension than body epidermis. (Evidence will be presented later to suggest that the corneal epithelium may derive both oxygen and nutriment by diffusion through its outer surface as well as from the aqueous humour.) Nevertheless, it will be shown that the corneal epithelium retains its distinctive properties when grafted on to or below body skin, and, conversely, that body skin epidermis survives as such when transplanted to the cornea.

METHODS AND RESULTS

Although experiments with guinea-pigs are referred to, the majority of those which gave indicative results were done on rabbits. All the operations to be described were done aseptically under ether anaesthesia.

The grafting of corneal tissue to the skin (Pl. 1, figs. 1–3). After steadying the eyeball by grasping a fold of loose conjunctival tissue at a single point with fine dog-toothed forceps, a 4–8 mm.^{2} square or rectangular 'Thiersch' graft may be cut from the cornea by pushing the point of a very sharp cataract knife very superficially and in an all but tangential direction into the stroma somewhat to one side of the anterior pole of the cornea. (Since the majority of the bundles of connective tissue fibres of the stroma form thin, multi-layered lamellae disposed parallel to the surface, the cornea 'splits' fairly easily at any desired level.) The shallow blind pocket so formed may be enlarged by a gentle to-and-fro motion of the blade until tissue of the required area has been lifted from the surface. The three attached edges of the pocket are then cut through and the entire graft lifted off and placed inner side downwards in a Petri dish lined with filter-paper moistened with saline solution. The anterior chamber is not pierced by this method of cutting a graft and the success of the operation does, indeed, depend upon its remaining intact. The graft consists of a sheet of corneal epithelium underlain by a layer of stromal connective tissue of from four to six times

its thickness. In spite of its speed of healing, the graft donor area is still visible to oblique inspection after a week as a shallow depression on the corneal surface representing the defect of stromal tissue.

In order to allow of proliferation and epithelial outgrowth, such grafts have been transplanted to the centre of 9–16 cm.^{2} square or rectangular raw areas prepared from the skin of the right or left side of the rabbit's chest (Pl. 1, figs. 8, 9, 11), following the technique of dressing described in full detail by Medawar (1944). We have found it advisable to prepare the raw area 4 days before the graft is transplanted to it, in order to allow the blood supply of the graft bed to become enriched by capillary proliferation in the ordinary course of 'granulating'. Transplantation then involves no more than removing the dressings, placing the graft in a more or less central position in known relationship to the superficial thoracic vessels that overlie the panniculus carnosus, and then applying fresh dressings.

A study of six rabbits so operated upon shows that epithelial outgrowth from the corneal graft proceeds in the same manner and at the same rate as from Thiersch grafts of body or ear skin. Outgrowth may be slight or, because of the transparency of the epithelial sheet, imperceptible on first inspection at the 7th to 10th post-operative day, but the graft centre is easily recognizable as a raised, rounded and transparent button in the midst of the granulation tissue. It reaches its maximum between the 17th and 25th days post-operation, when the graft is surrounded, not always symmetrically, by an outgrowth of perfectly transparent corneal epithelium averaging from ten to thirty times the area of the original graft (Pl. 1, figs. 8, 9). The finest details of the dermal vasculature may be seen through the epithelial sheet as clearly as through a glass window (Pl. 1, fig. 9). In vertical section (Pl. 1, fig. 10) the corneal epithelium, not exceeding 0·008 mm. in thickness, showed the characteristic appearance of actively migrating epidermal epithelium, the long axes of the basal-layer cells tending to lie parallel to the plane of the integument. The cell packing remained perfectly compact, the outer surface was sharply defined and the superficial keratinized layers showed no hint of a tendency to flake. Corneal epithelium showed no tendency to invade the underlying newly formed fibrous tissue to form inwardly directed 'pegs'. The epithelial outgrowth of Thiersch grafts of body or ear skin under just the same conditions (Pl. 1, fig. 11) is completely opaque; the epithelium may reach or exceed 0·02 mm. in thickness and is bounded superficially by a multilayered flaky cuticle (Pl. 1, fig. 12).

The attachment of the corneal epithelium to the substratum is mechanically very weak: the lightest scraping will damage it and cause bleeding. With the contraction of the raw area combined with the ingrowth of body skin epithelium from its edges, the corneal epithelium rather rapidly disappears, and a month's persistence in each of the two rabbits allowed to survive so long is the most we have recorded. We believe that this disappearance is due to the invasive replacement of the one epithelium by the other. This interpretation is supported by the behaviour of corneal grafts of a slightly different type made upon four guinea-pigs. The corneal grafts were transplanted to the centre of small raw areas, hardly larger than the grafts themselves, cut from the centre of small pigmented 'marker' grafts earlier transplanted to white chest skin to help in the identification of the transparent corneal grafts. Primary healing, revealed by a first inspection after 10 days, was satisfactory

and the grafts were recognizable as small transparent corneal buttons; but the grafts ceased to be visible by the 20th day, by which time native epithelium had closed the raw area and undermined the grafts.

Corneal grafts transplanted subcutaneously. In each of two guinea-pigs a single corneal Thiersch graft was implanted below the skin of the dorsal midline of the head by means of a forwardly directed trochar insertion beginning at a small incision roughly midway between the bases of the ears. Both animals were killed after 4 months, and the two implanted grafts proved to be similar in every respect. Each had formed a hard, raised, palpable nodule about the size of a small pea in the subcutaneous fascia. The nodules were in fact cysts (Pl. 2, figs. 15–17) of a pearl-like transparency containing a clear watery fluid under considerable pressure. Sections show the cyst to be bounded externally by an epithelium showing the characteristic conformation of cells under persistent tension. So far as the polarity of the cyst can be made out, it appears to have formed 'externally', i.e. the original outer surface of the corneal epithelium is directed inwards. Accumulations of squame cells such as would be expected to accumulate within a cyst of body skin epidermis are wholly absent. The origin and nature of the cyst fluid is obscure, though it must be supposed to bear some special relationship to the physiological activity of the corneal epithelium.

The transplantation of body or ear skin epidermis to the cornea. Because of the special ease with which it may be cut and handled, and the greater thickness of its epithelial layer, we have found ear skin to be superior to general body skin for grafting to the cornea. Square Thiersch grafts of side $1\frac{1}{2}$–$2\frac{1}{2}$ mm. were cut by defining the outline of the graft with very light scalpel incisions on the dorsum of the ear and slicing off the area within the incisions as thinly as possible with a no. 11 Swann-Morton scalpel blade. Such Thiersch grafts are always invested by a layer of dermal collagen below the epidermis.

The insertion of such a graft into the cornea proved to be easy. The first stage was to prepare a flat, very shallow, pocket in a plane parallel to but just below the surface of the cornea, exactly as in the cutting of a corneal graft (Pl. 1, figs. 1–3). Into this pocket the skin graft, shaped to fit whenever it proved necessary, was simply slid, with the dermal side facing inwards (Pl. 1, fig. 4). Enclosed round three of its four margins and held down under light pressure from above by the outer flap of the corneal pocket, such grafts always healed securely, and we have no technical failures to report.

In two preliminary experiments in which pigmented guinea-pig's ear skin was transplanted in this manner to the eye, the corneal stroma became richly vascularized by vessels penetrating inwards from the limbus, so defeating the object of the experiment—to grow body skin in the physiological environment characteristic of the normal corneal epithelium. (In our experience, all but the most trivial surgical injuries to the guinea-pig's cornea are followed by vascular invasion and a mass migration of pigmented conjunctival epithelium over certain sectors of the cornea.) Autopsy and histological examination at 11 days post-operation revealed exuberant epithelial proliferation with copious exfoliation of cuticle, accompanied by a general traumatic inflammation of the graft bed (Pl. 2, fig. 22). 'Clear cells' (cf. Billingham, 1948, 1949), representing the cell bodies of dendritic cells, stand out boldly in section (Pl. 1, fig. 13).

Ear skin grafts transplanted to the eyes of each of five rabbits proved not to provoke vascular invasion of the cornea. The grafts were examined *in situ* and removed for histological examination after periods ranging from 3 to 6 weeks.

It was our theoretical expectation that the thin corneal flap superficial to the skin graft, being shut off by the impermeable, richly cuticularized skin graft from access to material diffusing from the aqueous humour, would wither and die, so exposing the healed skin graft on the surface of the eye. It is a matter of some physiological interest that this did not happen: the corneal flap persisted throughout, although bounded by a corneal epithelium of very much reduced thickness (Pl. 1, fig. 14; Pl. 2, figs. 19, 23). These results seem to us to suggest that the corneal epithelium may derive some of its nutriment and oxygen supply by diffusion through its outer surface—a conclusion reinforced by unpublished experiments by one of us (R.E.B.) in which the skin grafts were replaced by very much larger 'grafts' of tantalum foil.

In practice, it proved that the skin epithelium tended to make use of the pocket housing it to form a long, shallow, but open cyst (Pl. 1, fig. 14), the epidermis breaking through to the surface at the opening of the pocket (Pl. 2, figs. 22, 24). The formation of layers of flaking cuticle showed that the epidermis continued to keratinize in the usual way (Pl. 2, figs. 19, 23), but the epidermis layer thinned out considerably and showed the subdued mitotic activity characteristic of non-vascularized skin grafts (cf. Medawar, 1948). Hairs and sebaceous glands did not differentiate *de novo*.

The epidermal component of skin grafts does not persist indefinitely in the eye, but submits to invasive replacement by the native corneal epithelium—the exact converse of what happens to a corneal graft when transplanted to skin. Pl. 2, fig. 18 shows clearly the overgrowth of skin dermal collagen, easily recognizable by its staining properties and the size and mode of packing of its fibres, by corneal epithelium. It seems clear that under the physiological conditions prevailing in the cornea the native epithelium is at an advantage both in division rate and in migratory activity.

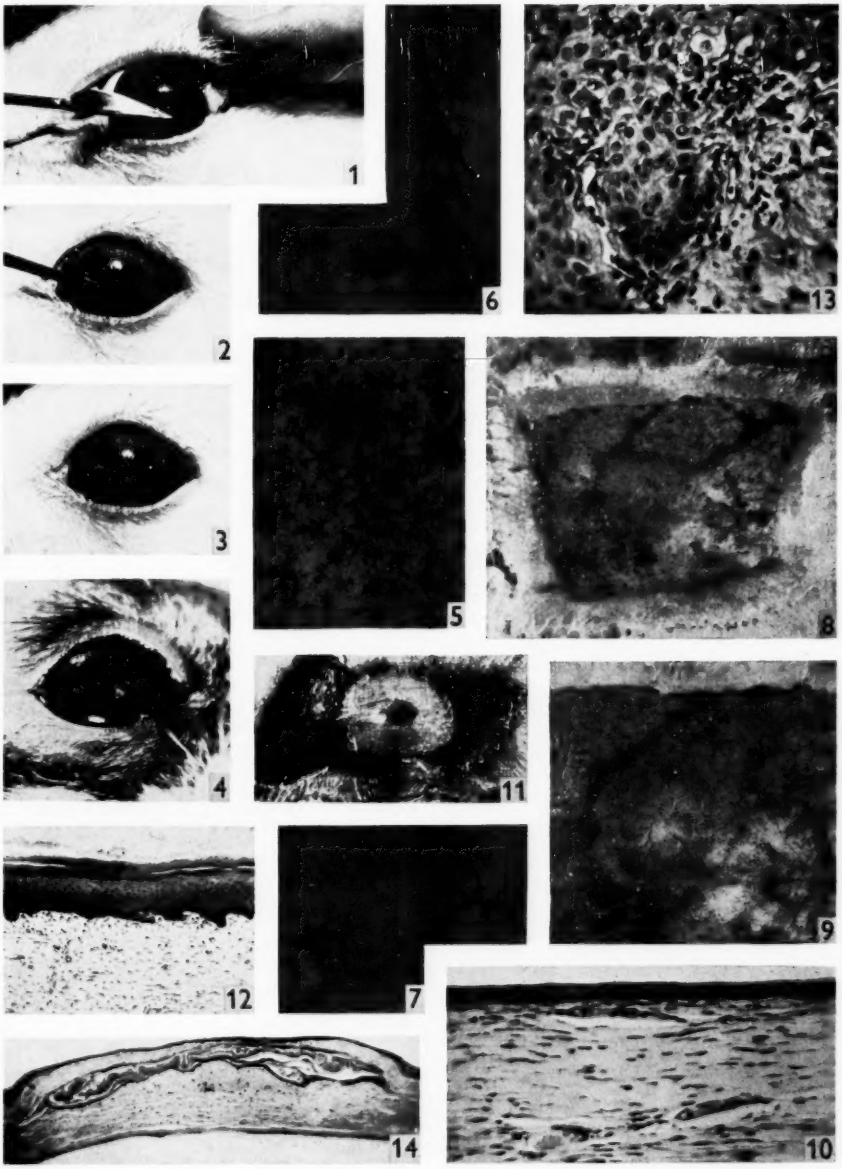
The experimental results recorded in this paper make it clear that the peculiar histological differences between adult corneal and body-skin epithelium are inherited or 'genetic', and are not to be attributed to the physiological differences between the environments in which they normally live. The subdivision of the genus 'epidermal epithelium' into its several genetic species—the epithelia of sole skin, body skin, cornea, tongue, claw, etc.—reinforces our belief that such a hierarchical classification is peculiarly appropriate to the cells of the body, a matter already discussed elsewhere (Billingham & Medawar, 1948*b*). We believe that the classification of the cell lineages of the body is an essential part of descriptive embryology and an indispensable preliminary to understanding the mechanism of embryonic differentiation.

SUMMARY

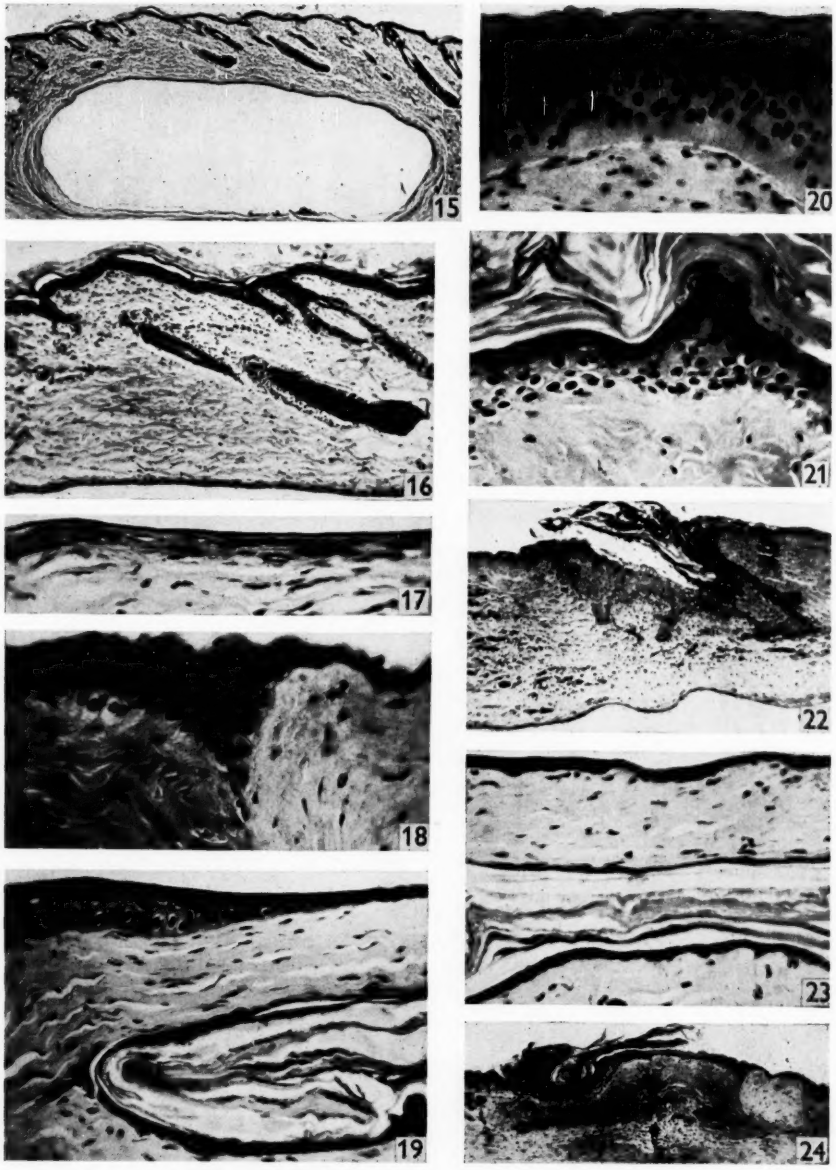
1. Operations are described by means of which corneal epithelium may be transplanted to body skin and body skin may be transplanted to the cornea.

2. Both epithelia retain their specificity of histological type in a physiologically and anatomically foreign environment.

3. Corneal epithelium is invasively replaced by skin epithelium when transplanted



BILLINGHAM AND MEDAWAR—THE SPECIFICITY OF THE CORNEAL EPITHELIUM



BILLINGHAM AND MEDAWAR—THE SPECIFICITY OF THE CORNEAL EPITHELIUM

to the skin, and skin epithelium is invasively replaced by corneal epithelium when transplanted to the cornea.

4. The subdivision of the genus epidermal epithelium into its several genetic species is discussed.

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REFERENCES

Billingham, R. E. (1948). Dendritic cells. *J. Anat., Lond.*, **82**, 93–109.

Billingham, R. E. (1949). Dendritic cells in pigmented human skin. *J. Anat., Lond.*, **83**, 109–115.

Billingham, R. E. & Medawar, P. B. (1948*a*). Pigment spread and cell heredity in guinea-pigs' skin. *Heredity*, **2**, 29–47.

Billingham, R. E. & Medawar, P. B. (1948*b*). 'Infective' transformations of cells. *Brit. J. Cancer*, **2**, 126–131.

Medawar, P. B. (1944). The behaviour and fate of skin autografts and skin homografts in rabbits. *J. Anat., Lond.*, **78**, 176–199.

Medawar, P. B. (1948). Immunity to homologous grafted skin. III. The fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. *Brit. J. exp. Path.* **29**, 58–69.

Redslob, E. (1922). Étude sur le pigment de l'épithelium conjunctival et cornéen. *Ann. Oculist., Paris*, **159**, 523–537.

EXPLANATION OF PLATES

Plate 1

Figs. 1–3. Illustrating the technique of preparing pockets in the cornea, or alternatively for cutting corneal Thiersch grafts. For description, see text. In fig. 2 a paper strip has been inserted into the pocket to reveal its length and depth.

Fig. 4. A small rabbit's ear skin Thiersch graft 23 days after transplantation into the type of pocket illustrated by fig. 3. Note the absence of any trace of corneal opacity or vascularization.

Figs. 5–7. The 'peculiar branched wiry bodies' immediately below the corneal epithelium revealed by supravital staining with methylene blue. In fig. 5 ($\times 80$) they stand out at a different focal plane from the more diffusely stained stromal branched cells. In figs. 6 and 7 ($\times 334$) note the relationship of the bodies to the just perceptible outlines of the basal layer cells of the corneal epithelium. These bodies are not dendritic cells. The preparations were made from the cornea of the ox and were rendered permanent by the ammonium molybdate method.

Figs. 8 and 9. Showing the widespread outgrowth from rabbit corneal Thiersch grafts transplanted to raw areas cut from the skin of the chest. Fig. 8 ($\times 1$) shows the boundaries of the outgrowth; fig. 9 ($\times 1\frac{1}{2}$) shows the original graft as the small rather dense central patch. Notice that the pattern of the dermal vasculature can be made out in the finest detail through the perfectly transparent outgrowth of corneal epithelium.

Fig. 10. A transverse section through the outgrowth of corneal epithelium illustrated by figs. 8 and 9. Note the characteristic packing of the cells and the complete absence of a desquamating cuticle. Ehrlich's haematoxylin and eosin, $\times 210$.

Fig. 11. Contrast figs. 8 and 9. Widespread symmetrical outgrowth of opaque desquamating epidermal epithelium from a rabbit's ear skin Thiersch graft 20 days after transplantation to a raw area prepared from the skin of the chest. The central graft is darker and has grown a sparse pelt of hairs since transplantation, $\times 1\frac{1}{4}$.

Fig. 12. A transverse section through the outgrowth of ear skin epidermis illustrated by fig. 11: contrast fig. 10. The epithelium is hyperplastic and strongly exfoliating. Ehrlich's haematoxylin and eosin, $\times 50$.

Fig. 13. (See Pl. 2, fig. 22.) Guinea-pig ear skin 11 days after transplantation to the cornea, showing Malpighian cells and clear cells. The cornea has become vascularized, and shows inflammatory changes. Ehrlich's haematoxylin and eosin, $\times 216$.

Fig. 14. (See also Pl. 2, figs. 19 and 23.) A cyst of rabbit's ear skin epithelium 23 days after transplantation to a corneal pocket such as that illustrated by fig. 3. Note the thinness of the epithelium, the accumulation of cuticular debris within the cyst, and the persistence over the cyst of an intact layer of corneal epithelium of reduced thickness. The dermal collagen of the skin graft is still recognizable. Ehrlich's haematoxylin and eosin, ×38.

PLATE 2

Figs. 15–17. Cyst of guinea-pig's corneal epithelium 126 days after transplantation below the skin of the dorsal midline of the head. The cyst, originally fluid filled and containing no cellular debris, is bounded throughout by a thin, 'taut' layer of corneal epithelium, illustrated in higher power by figs. 16 and 17. Ehrlich's haematoxylin and eosin; fig. 15, ×15; fig. 16, ×51; fig. 17, ×210.

Fig. 18. A view in higher power than fig. 24 of the junction between corneal stroma and skin dermis in a rabbit's ear skin Thiersch graft 43 days after transplantation to the cornea. The skin dermal collagen to the left can be distinguished from the corneal stroma to the right by the deeper staining of its fibres, their greater stoutness, and their three-dimensional mode of packing. Both connective tissue elements are bounded above by *corneal* epithelium, which has invasively replaced the skin epithelium. Ehrlich's haematoxylin and eosin, ×210.

Fig. 19. Transverse section of the margin of the cyst illustrated by Pl. 1, fig. 14 (rabbit's ear skin 23 days after transplantation to a corneal pocket). Note that the corneal epithelium on the outer surface of the eye still survives in progressively diminishing thickness over the epithelial cyst, although virtually deprived of access to materials diffusing from the aqueous humour. See also fig. 23. Ehrlich's haematoxylin and eosin, ×210.

Figs. 20 and 21. Contrasting the appearance of hyperplastic corneal epithelium (fig. 20) with ear skin epithelium (fig. 21) 43 days after the transplantation of rabbit's ear skin to the cornea (see also fig. 24). The pyknotic appearance of the nuclei in fig. 21 is a characteristic of skin growing under conditions of reduced oxygen tension. Contrast the richness of exfoliating cuticle in fig. 21 with its absence in fig. 20. Ehrlich's haematoxylin and eosin, ×210.

Fig. 22. Cf. Pl. 1, fig. 13. General sectional view of a graft of guinea-pig's ear skin 11 days after transplantation to the cornea, which has since become richly vascularized. The section passes vertically through the edge of the corneal pocket into which the skin graft was originally tucked, and exfoliating epidermal epithelium has broken through to the surface of the cornea. Ehrlich's haematoxylin and eosin, ×44.

Fig. 23. A view in higher power of a vertical section through the middle of the cyst shown in Pl. 1, fig. 14, one formed by rabbit's ear skin epithelium 23 days after transplantation to a pocket in the cornea. The corneal epithelium that bounds the specimen above is still surviving, though of reduced thickness (cf. fig. 19). The skin epithelium bounding the cyst within the corneal stroma consists of only one or two cell layers. Ehrlich's haematoxylin and eosin, ×210.

Fig. 24. Cf. also figs. 18, 20, 21. A general sectional view of a rabbit's ear skin graft 43 days after transplantation to the cornea. Skin dermal collagen may be recognized by the criteria mentioned in the legend to fig. 18. The non-exfoliating corneal epithelium is invasively replacing the body skin epithelium from right to left: see fig. 18. Ehrlich's haematoxylin and eosin, ×38.

REVIEWS

A Cyto-architectonic Atlas of the Rhombencephalon of the Rabbit. By H. MEESSEN and J. OLSZEWSKI. (Pp. 52; 15 text-figures and 15 plates; S. fr. 50.) Basel and New York: S. Karger.

This fine atlas is illustrated by excellent microphotographs of transverse sections showing the cyto-architectonics of the medullary and pontine regions of the rabbit's hind-brain. The authors undertook the preparation of the atlas in order to provide more accurate topographical data than are available in the well-known atlas of Winkler and Potter. With Rose's atlases of the cerebral cortex and the thalamus, and Gillilan's atlas of the mid-brain, the details of the cyto-architectonics of the rabbit's brain have now been assembled fairly completely, and they will certainly be of considerable value to the anatomist and physiologist in the experimental study of problems of localization of brain functions in this laboratory animal. The descriptions of the microphotographs in this new atlas are short and explicit and are given in double columns, German and English.

W. E. LE GROS CLARK

Functional Localization in the Frontal Lobes and Cerebellum. By J. F. FULTON. (Pp. 140; 53 text-figures; 15*s*.) Oxford: Clarendon Press.

The publication of the William Withering Memorial Lectures given by Dr Fulton at Birmingham in 1948 is particularly welcome, since it provides, in summary form, a review of the more recent work on functional localization in the frontal lobes and the cerebellum, including a useful bibliography. Functional localization in the frontal lobe is being intensively studied at present in connexion with two main problems—the precise significance of the respective roles of the motor and 'premotor' cortex in voluntary movements, and the interpretation of the clinical results observed after the operation of leucotomy. These problems, which form the substance of the first three lectures, are discussed by the author in a stimulating manner, and he makes it abundantly clear that evidence is now accruing which suggests a much more precise type of functional localization than had been supposed. The last lecture deals with the question of cerebellar localization, in which special reference is made to the physiological studies of Adrian and the anatomical studies of Dow. Mention is also made here of recent experiments by Nulsen and his collaborators, which suggest that it is possible to map the entire anterior lobe of the cerebellum in terms of the representation of individual muscle groups. Here, again, it seems that the precision of functional localization is likely to exceed previous expectation. This book is excellently produced, and is illustrated with very effective diagrams.

W. E. LE GROS CLARK

The Temporal Bone and the Ear. By THEODORE H. BAST, A.B., PH.D., and BARRY J. ANSON, M.A., PH.D. (Pp. xviii + 478; 166 figures; med. 8vo; 60*s*. net.) Springfield, Illinois: Charles G. Thomas; and Oxford: Blackwell Scientific Publications Ltd. 1949.

This is a well-produced, well-printed and well-illustrated book, but the title is misleading, for the main subjects of the book are the development of the otic and periotic labyrinths and of the otic capsule.

The first chapter, on 'The Temporal Bone and the Ear', is the least satisfactory. It is illustrated by well-known diagrams from Gray's *Anatomy* and Cunningham's *Dissecting Manual*, some of which contain typographical errors copied from the originals. It is not a particularly good account of the temporal bone and the ear and it is a curious chapter to find in a book of this kind.

The remainder of the book is of a very different character. It contains the results of careful work on a large amount of material, is full of interesting information and is illustrated by excellent photographs.

The book is essentially developmental in outlook and the three chapters, on the otic labyrinth, the periotic labyrinth and the otic capsule are all very good. There is an interesting account of the endolymphatic duct and sac, and of the development of the cochlea which is described in an unusual, though correct, orientation illustrated by a rather curious figure. It is good to find the terminology of the otic and periotic labyrinths cleared up.

The book is curiously uneven, for example, 20 pages are devoted to describing the fissula ante fenestram, 12 pages to the process of normal ossification. There is a long (80 pp.) and interesting account of the ossification of the otic capsule, with 12 pages devoted to an historical account of its ossification centres.

There is an interesting section dealing with otosclerosis and its relation to the fissula.

There is no special account of the organ of Corti as its 'detailed histology is well described in most text-books'.

In the section on the middle ear and its related air spaces the latter are dealt with in considerable detail and their development is well illustrated, but the tympanum as a whole is not discussed at any length. The muscles, the malleus and the incus receive little attention, while almost the whole of the chapter on the ossicles deals with the stapes, and very well too.

The book concludes with a most interesting historical survey of the structure and functions of the internal ear, in which, however, emphasis is laid on function.

The book is well produced, the paper and print both being very good. There are only a few misprints. The illustrations are an outstanding feature of the book, but they would be easier to follow and more valuable if the legends were simplified. At present these begin with an unnecessary general statement of the subject of the figure, then the magnification, then the length in mm. and the age in weeks of the specimen from which the section was taken, then the source of the specimen—Harvard, Wisconsin, or wherever it may be, then the serial number of the specimen, the number of the slide and the number of the section, until at last one arrives at the legend that tells one what is what in the figure. It would be a great improvement if the serial details and magnification could be put after instead of before the legend.

The copy under review is not bound as well as it might be, and there appears to be some danger of the pages becoming loose after much use.

The book is a mine of information on the early developmental stages of the internal ear, with many interesting historical touches. The authors are to be congratulated on the research which has led to the production of this useful and pleasing book.

C. M. WEST

Textbook of Histology. By J. F. NONIDEZ and W. F. WINDLE. (452 pages, 209 drawings and 193 photomicrographs; 1949.) McGraw-Hill Book Co.

This book, written for medical and dental students in the early stages of the pre-clinical courses, is refreshing to read and very well illustrated. It would be easy to criticize it for lack of descriptive detail, but the authors have set out to condense the ever-growing factual side, to simplify terminology as far as possible, and to aid the beginner in grasping essentials. Unfortunately, the time-worn pattern of the standard texts is retained and, although it is claimed that a new approach to the teaching of histology is presented, there is actually little that is new beyond references to films which are suitable for showing in conjunction with the text. The descriptive side of histology is poor fare for the modern student well-grounded in physics and chemistry and, although we cannot dispense with mere description, it is essential, if interest is to be maintained, to emphasize the implications of histology in the study of physiology, biochemistry, pharmacology and pathology, and to stress the quantitative and experimental aspects where possible. One looks almost in vain in this book for the odd phrase or sentence giving a clue to the importance of a particular cell-type

in the aetiology of some pathological condition. The links with physiology are put in at random, whereas in most cases they should be the high-lights in the description of a particular organ or tissue. It is indeed almost incredible to find no reference to innervation, and the structural changes following stimulation, in the sections dealing with salivary glands, stomach and pancreas. Countless other opportunities have been missed, as though the authors were hesitant in encroaching on the fields of physiology and embryology. Too often one is left with the feeling, as with many larger text-books, that the subject is being presented for its own sake in isolation, and not as part of the biology of the mammalian body. One queries also whether it is advisable to describe all the components of cells in the second chapter of the book, and then to devote the third to epithelia and glands. The student at the outset is not in a position to assimilate summarized information, and he would be much better served if introduced as early as possible to some composite part of the body so that he can appreciate how cells of very varied architecture co-operate in forming one region or another. The fine details of cells and epithelia would form an admirable summary at the end of the book when the variations in size, shape and structure of nuclei or mitochondria will have been seen for what they are worth and not regarded as equally important for all the cells of the body. The references given for additional reading have been chosen from papers published mainly in the last few years. While these may illustrate where contemporary interest is focused, such a selection can have grave disadvantages. For instance, the chapter on the mammary gland concludes with a paper on the apocrine sweat glands of the breast—an 'interesting side-light' but rather atypical of the modern literature on this organ. It is indeed difficult to write a simplified text-book on a pre-clinical subject, and some may think that it is better to train students to use larger ones so that they learn the indispensable task of selecting relevant information and discarding what is unnecessary for their purpose.

K. C. RICHARDSON

PROCEEDINGS OF THE ANATOMICAL SOCIETY

NOVEMBER 1949

The Annual General Meeting of the Society, for the Session 1948–49, was held on Friday, 26 November 1948, in the Department of Anatomy, Middlesex Hospital Medical School, Mortimer Street, London, the President (Prof. A. B. Appleton) in the Chair.

The following are the authors' abstracts of the papers read:

The relative sizes of limbs and jaw in man. By R. H. Shepherd, D. A. Sholl and A. Vizoso. *Department of Anatomy, University College, Gower Street, London, W.C.* 1

Measurements of height, lengths of upper and lower limbs and jaw were made on a large number (*c.* 800) of males whose ages ranged from 2 days to 20 years. It is found that the regression of the length of the part on the total body length is linear and that no evidence of allometric growth in the sense of Huxley and Teissier is found at any stage. A method is given to show how these data may be used as norms for the early detection of faulty skeletal development.

The distribution of the lacrimal fluid. By E. Wolff
46, Wimpole Street, London, W. 1

The tear fluid normally collects in the superior and inferior marginal strips which lie in the grooves where the lid margin touches the globe, and in the *outer* tear lake at the *outer* canthus. There is practically no fluid in the so-called lacus lacrimalis. These collections of fluid form a reservoir which is prevented from overflowing by the oily secretion of the Meibomian glands. The fluid will therefore be guided to the puncta mainly along these collections. None flows over the eye as usually described. The puncta dip into the marginal strips and not into the lacus lacrimalis, which really contains no fluid for them to drink.

The junction of skin and conjunctiva at the lid margin is at the junction of dry and moist portions, i.e. at the posterior margin of the openings of the Meibomian glands where the lid margin is no longer wetted by the tears.

The precorneal film consists of three more or less distinct layers—oily, lacrimal and mucous from superficial to deep.

The development of the mammary gland (early intra-uterine phase). By E. S. R. Hughes. *Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.* 2

The precursor of the mammary gland is a cord of epithelial cells, the 'milchleiste', mammary ridge, or milk line, which can be traced from the axillary region to just distal to the umbilicus; caudally it is continued into the inguinal region in the form of a more highly differentiated ectoderm.

This epithelial cord rapidly shortens and in the 30 mm. embryo the anlage has become globular in shape. This shrinkage appears to be due partly to desquamation of the epithelium and partly to its atrophy after it has become buried in the mesoderm. As the mammary ridge shortens, it rotates from a dorsal to a ventral position.

The epithelial anlage possesses a germinal layer of tall columnar cells, with clear cytoplasm and apical nuclei. The remaining cells are rounded, cubical or flattened, with deeply staining oval nuclei.

The mesenchyme deep to the epithelial cord of the 7·1 mm. embryo is rather more dense than elsewhere in the subectodermal area; by the 13 mm. stage a mesodermal capsule of spindle-shaped cells has become closely applied to the basement membrane of the epithelial ingrowth; whilst in the 30 mm. embryo three zones can be distinguished, the mesodermal capsule now consisting of several laminae, a deep layer of thick fascia and an intermediate dense, vascular zone.

Collagen and pre-collagen. By G. H. Bourne. *Department of Anatomy, London Hospital Medical College, Turner Street, London, E.* 1

All adult collagen appears to pass through a pre-collagenous stage in the embryo. Even in the regeneration of soft tissues or of bone the first-formed fibres are of a pre-collagenous or reticular nature. Under abnormal conditions (e.g. scurvy) the change of pre-collagen fibres into collagen may be delayed, or under other circumstances (e.g. a burn) collagen may revert to pre-collagen. This interconvertibility between collagen and pre-collagen suggests that the difference between the two types of fibres is a physical one. Evidence from various sources suggests that collagen fibres are made up of protein chains or aggregates in the form of submicroscopic rodlets or micelles. An arrangement of these micelles is suggested which helps to explain the differences between the staining reactions of the two types of fibre and their ready interconvertibility.

Diffusion phenomenon complicating the histochemical reaction for alkaline Phosphatase. By B. F. Martin and F. Jacoby. *Department of Anatomy, University College, Newport Road, Cardiff*

In the technique employed for the histochemical demonstration of alkaline phosphatase the incubation time recommended varies within wide limits, some workers incubating the sections for 2–3 hr., others for as long as 24 hr. The intensity of the reaction increases with the incubation time, and some structures that are negative at a short incubation period may give a positive reaction after a longer one. This is particularly the case with nuclei and cells near regions of intense phosphatase activity. Such findings make it difficult to localize the true sites of the enzyme and give rise to the suspicion that some of the phosphatase reaction may be artificially brought about by a process of diffusion (namely, of enzyme or its reaction products) from areas of intense activity.

This problem has been investigated in two ways:

(*a*) Sections of a variety of tissues known to have a high phosphatase activity were superimposed on ready-mounted sections of tissues known not to have such activity. After incubation, sometimes for even only half an hour, nuclei in the lower section that lay close to areas of high phosphatase activity in the upper showed a positive reaction.

(*b*) From unmounted paraffin sections of rabbits' duodena small wedges containing portions of Brunner's glands were excised, mounted separately from the remainder of the sections, and treated in the usual way. Even after 3 days' incubation the Brunner's glands in these wedges showed no reaction, while those in the parent material all showed a positive nuclear reaction after 24 hr. incubation or even earlier. It should be added that only such duodena were used which when investigated gave a widespread nuclear reaction.

These experiments show that a positive reaction can be obtained in areas of no inherent phosphatase activity by diffusion from areas of intense activity; and that this process of diffusion complicates, in no small measure, the evaluation of the reaction and has to be guarded against.

The fate of an intra-neural crystalloid injection as demonstrated by the radio-active tracer technique. By J. B. Brierley and E. J. Field. *Department of Anatomy, The University, Bristol* 8

The widespread employment of intra-neural injections in the experimental investigation of such diseases as tetanus, rabies and poliomyelitis calls for an investigation of the potential spread of an indifferent indicator in the nerve and spinal cord as well as in the blood and cerebro-spinal fluid.

0·05 m. of radio-active phosphorus (50–60 μc.) was injected into the sciatic nerve of sixteen rabbits; in four at the popliteal fossa ('low sciatic' injections), in six at the mid-femoral level ('mid-sciatic' injections) and in six at the edge of the pelvis ('high sciatic' injections). A series of four control animals received 50–60 μc. of P_{32} intravenously in order to provide information as the extent of entry of P_{32} into the various segments of the sciatic nerve and spinal cord from the blood stream.

Specimens of whole blood were removed for radio-active estimation at intervals of 10–15 min. Four to five samples of cerebro-spinal fluid were removed by cisternal puncture during the survival period of 2 hr.

All the intra-neural injections could be resolved into two groups. In the first there was a rapid absorption into the blood producing counts up to 150 q./min./100 mg. and the indicator had moved on a few millimetres along the nerve. The activities of cerebro-spinal fluid and spinal cord were lower than those of the controls. In the second group the blood curves were much lower, while the activity of the cerebro-spinal fluid sometimes exceeded 2500 q./min./100 mg. There was a direct spread of the indicator along the nerve in some cases throughout the spinal cord and brain stem up to the basal ganglia. Injections of this ('cerebro-spinal fluid') type were associated with minimal plunger resistance, while a column of fluid was seen to run rapidly up to the nerve at the instant of injection—features not observed in injections of the first ('blood') type.

In thirteen out of sixteen cases the opposite sciatic nerve showed activities considerably in excess of the control level and in eight of these thirteen the region of increased activity showed a rough correspondence to the site of injection.

An attempt was made to determine the anatomical distinction between the two types of injection by repeating the procedure with indian ink as the injection mass. Here again the two types of injection were encountered and transverse celloidin sections of the nerves showed that in injections of 'cerebro-spinal fluid' type the ink had ascended the nerve as a narrow black column, the particles streaming within the fasciculus in the inter-fibre spaces.

Argentaffin dendritic cells in hair follicles. By J. D. Boyd. *Department of Anatomy, London Hospital Medical College, Turner Street, London, E.* 1

Silver techniques reveal the existence of an extensive system of dendritic cells in the hair follicles of Man. Similar cells have also been found in the hair follicles of the pig, the rabbit, the rat, the mouse and the shrew. In the human embryo the cells were first convincingly found at the 150 mm. stage but some evidence of their presence at earlier stages is available. They first differentiate in the hair follicles of the eyebrow, the upper lip and the chin. The possible relation of the cells to hair pigmentation is discussed.

The popliteus muscle and the lateral meniscus. By R. J. Last. *Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.* 2

The description of the popliteus as having no attachment to the lateral meniscus is faulty. Upwards of half of the muscle belly is attached to the posterior arch of the lateral meniscus and has no insertion into the femoral tendon of attachment. It is suggested that herein may lie the true explanation of the relative immunity of the lateral meniscus to injury. The lateral meniscus is further attached, by its posterior horn, to the femoral condyles by the ligaments of Humphry and Wrisberg. The latter structures are unrecognized in B.N.A. and Birmingham revision terminology; yet they may exercise an indispensable function in controlling the position of the lateral meniscus. Injuries of the menisci occur in rotating the partially flexed knee. The suggestion is made that in rotation the position of the lateral meniscus is under ligamentous and muscular control, and that the absence of such control is the reason for the preponderance (10:1) of laceration in the case of the medial meniscus. In lateral rotation of the femur on the tibia the ligaments of Humphry and Wrisberg draw the posterior arch of the lateral meniscus medially, but at the same time the popliteus draws the arch downwards over the bevelled posterior edge of the tibial condyle. This carries it in advance of the lateral femoral condyle as the latter moves backwards. The return of the meniscus to the position of rest is due to elastic recoil as the ligaments of Humphry and Wrisberg and the popliteus muscle relax.

Experiments on the hypophysial portal vessels. By G. W. Harris. *Department of Physiology, University of Cambridge*

The theory that the hypothalamus influences the secretory activity of the anterior pituitary gland by means of chemo-transmission of stimuli through the hypophysial portal vessels rests on the assumption that the blood flows in these vessels *from* the tuber cinereum

to the pituitary gland. Popa & Fielding (1930), who first described the anatomy of these vessels, held that the blood flowed from pituitary gland to brain. Since that time much evidence of an indirect nature has been adduced which indicates that the flow is towards the pituitary gland. In collaboration with Dr J. D. Green a technique was devised for viewing the pituitary stalk of living anaesthetized rats under the microscope (Green & Harris, 1948). In all animals we observed the flow to be from the median eminence of the tuber cinereum to the pars distalis of the pituitary.

There are many claims in the literature that normal anterior pituitary function may follow pituitary stalk section. The conclusion is often drawn that the hypothalamus does not therefore influence the adenohypophysis via the pituitary stalk. Forty-five rats, in which the pituitary stalk had been sectioned some time previously, were killed and their vascular systems perfused with indian ink solution. A block of tissue containing the hypothalamus and pituitary gland was, in each case, serially sectioned in celloidin. It was found that in the majority of animals the portal vessels had regenerated across the site of the stalk section. Much of the work on stalk section in relation to anterior pituitary function should be repeated and the state of the portal vessels controlled by indian ink injections. It seems possible that the varied results reported to follow such section may be accounted for by varying degrees of regeneration of these vessels.

REFERENCES

Green, J. D. & Harris, G. W. (1948). Submitted for publication in *J. Physiol.*
Popa, G. T. & Fielding, U. (1930). *J. Anat., Lond.*, **65**, 88.

The renal shunt mechanism and anoxia (preliminary note). By K. J. Franklin.
Department of Physiology, St Bartholomew's Hospital Medical College, Charterhouse Square, London E.C. 1

In the anaesthetized rabbit anoxia, produced by occlusion of a cannula tied into the trachea, causes, after a minute or two, a diversion of the blood flow away from the outer layers of the renal cortex, and hence paling of the kidney surface. Simultaneously, there is a rise of arterial blood pressure, partial constriction of the renal arteries, a fall in kidney volume, and reduction or cessation of urine flow. The renal effects are prevented by previous denervation of the renal vessels, i.e. they are nervously intermediated, and such extra adrenaline or other hormone as may be liberated during the anoxia plays no obvious part in the changes observed. Recovery from the effects is more rapid than their onset, and the whole cycle can be repeated several times at short intervals.

The initiation of the reflex is associated with strong inspiratory effort, and mere over-inflation of the lungs through the tracheal cannula produces similar effects. The vagi in the neck can be divided without affecting the results of such inflation. The pathways concerned are being further investigated, with Prof. E. C. Amoroso as collaborator.

Administration of carbon monoxide affects the kidney in much the same way as tracheal occlusion, except in so far as blood colour is concerned. The concentration of gas required, however, makes subsequent recovery impossible, so the experiment cannot be repeated.

In so far as they have gone, observations on the dog and the cat correspond with those on the rabbit. So anoxia, produced as described, shunts the renal blood away from the renal cortex.

Chronic experiments with carbon monoxide have not justified the hope that the periodic administration of this gas would produce hypertension and so provide a new approach to the solution of the clinical problem.

Some of the work described was done in collaboration with Prof. E. C. Amoroso, Miss E. A. Ullmann, and Dr L. E. McGee.

Secondary olfactory connexions in monkeys. By A. C. Allison and M. Meyer.
Department of Anatomy, University of Oxford

The secondary olfactory connexions were studied experimentally in five monkeys (*Macaca* and *Papio*) by following with a silver technique the degeneration of fibres and terminals resulting from transection of the olfactory peduncle on one side. It was found that fibres in

the olfactory tract terminate in the prepiriform area (mainly on the temporal lobe), in the cortico-medial group of amygdaloid nuclei, and the rostro-lateral part of the olfactory tubercle. There is an extensive bilateral distribution of olfactory fibres in the anterior limb of the anterior commissure to the bed nucleus of the stria terminalis, part of the medial preoptic region and the central amygdaloid nucleus. Very few, if any, olfactory fibres reach the anterior hippicampal nucleus and septum.

Observations on ascending and descending tract degeneration following midbrain tractotomy in the human. By P. Glees, D. R. Oppenheimer and R. A. Bailey. *Department of Physiology, University of Oxford*

Knowledge of the course and position of fibre tracts in the brain stem is based mainly on animal experiments. However, new developments in human brain surgery in recent years have made it possible to study in post-mortem material degenerating brain tracts caused by small lesions. One of these surgical methods is tractotomy for incurable pain. A midbrain tractotomy was done by one of us (Bailey) and observations on the position and course of the following systems were made:

Ascending systems: (1) medial fillet; (2) spino-thalamic and quinto-thalamic tracts; (3) lateral fillet; (4) supraoptic and posterior commissures.

Descending systems: tegmento-olivary tract.

Further evidence is given of the distribution of primary trigeminal fibres in the pons and medulla.

The reference of pain aroused in the skin. By D. C. Sinclair. *Department of Human Anatomy, University Museum, University of Oxford*

In the study of the 'reference' of pain from one part of the body to another, little attention has been paid to the possibility that stimulation of one area of skin might, under certain circumstances, give rise to pain referred to another. A number of observations have been made on the occurrence of this phenomenon in young, healthy adults, and it has been found that stimulation of certain randomly distributed discrete points in the skin may cause a fairly well localized stinging sensation, either in another part of the territory of the same spinal segment, or in an area of skin having no apparent neural relation to the point stimulated. Pain appears to be the only sensation which can be referred in this way and it is accompanied by changes in skin temperature and sensibility in the area of reference. The mechanism involved may perhaps be related to the process postulated to occur in cases of referred pain from deeper structures by Sinclair, Weddell & Feindel (1948). This theory depends on the existence of branching among the axons conveying the sensation of pain to the central nervous system, this branching being of such a type that one limb of the axon passes to the site of origin of the disturbance, while others pass to the sites to which the pain is referred.

The neural pattern of the epiglottis. By W. H. Feindel. *Department of Human Anatomy, University of Oxford*

The pattern of innervation of whole preparations of the epiglottis from rabbits and monkeys has been studied by the aid of intravenous methylene blue staining.

The posterior surface receives a dense innervation derived from bundles of nerve fibres which enter at the base. The fibres from any one bundle ascend and ramify over a wide area, interlapping to a great extent with fibres from other bundles. Conspicuous giant hederiform endings, which are supplied by large myelinated fibres, can be identified in the complex network of other fibres and endings and provide a key to the neural pattern. Any small region of the posterior surface is innervated by a group of endings, of the same morphological type, supplied by axons which approach that region from different directions and by way of different nerve bundles.

The anterior surface, in contrast, is sparsely innervated by non-myelinated, freely ending fibres.

The effect of pressure on conduction and nerve fibre size. By G. CAUSEY.
Department of Anatomy, University College, Gower Street, London, W.C. 1

The effect of direct pressure by mercury on the nerve to the medial head of gastrocnemius in the rabbit was investigated. It was found that, although pressures of 80 mm. Hg and over produce diminution of the nerve bundle size, and the mean fibre size, pressures of 125 mm. Hg and over were required to cause block of conduction to the muscle when a stimulus was applied proximal to the site of pressure. The time necessary to block varied between 6 and 36 min. Recovery of conduction occurred in all cases within 60 sec. after pressure was released and air admitted around the nerve. Recovery of nerve fibre size and nerve bundle size was demonstrated histologically within 10 min. of the release of pressure. It was shown that after the aorta and pulmonary arteries had been ligated at the base of the heart, local pressure could produce block, and its release recovery of conduction before death of the muscle nerve preparation. Local pressure on the nerve trunk by air at a pressure of 240 mm. Hg did not produce block in 52 min.

It is concluded that blockage of conduction in acute experiments is due to local anoxia produced by the pressing out, from the compressed region, of blood and endoneurial fluid.

The nervous constituent of the atrio-ventricular bundle. By E. J. FIELD.
Department of Anatomy, The University, Bristol 8

The nervous component of the bundle of His has been studied in the sheep, rabbit, chimpanzee, rat and man chiefly by the Bielschowsky-Gros method for frozen sections. In the sheep the nerves of the bundle are prominent and numerous but do not expend themselves in its innervation. Bundles of nerve fibres course for considerable stretches beneath the endocardium of the interventricular septum before turning into the depths of the myocardium. Fibres depart at intervals from the branches of the bundle to pass into the general myocardium. No indisputable nerve endings were found either in the cells of the atrioventricular bundle or in the ordinary heart muscle. Numerous nerve fibres accompany many—but not all—of the branches of the coronary arteries. No nerves were found in the media of these arteries. The existence of a fine network of fibres passing between neighbouring capillaries—as figured by Ph. Stöhr, jr.—was confirmed, but no nerve endings on capillary walls were made out. In the human the nervous component of the atrioventricular bundle is less prominent and even less so in the rabbit and monkey. In these latter animals the septal muscular connexion between the atria and ventricles does not serve also as an avenue for nervous connexion. In the rat, however, where the bundle of His is a comparatively poorly developed structure, numerous nerve fibres utilize it as a high road between the atria and ventricles. They do not, however, bear a particularly close relation to the bundle, and after reaching the top of the interventricular septum they pass off to right and left without obvious relation to 'specialized' tissue. The existence of these nervous connexions between atria and ventricles—though recognized by Tawara himself—do not appear to have received adequate recognition in the formulation of modern theories of heart action.

Growth of nerve implants in muscle. By J. T. AITKEN. *Department of Anatomy, University College, Gower Street, London, W.C.* 1

Nerve fibres were made to grow into a nearby muscle by implanting the cut end of the nerve in the muscle. If the host muscle had been previously denervated, the regeneration nerves produced motor end-plates in the region of the implant and for some distance beyond. These end-plates were shown to be functional by stimulation of the nerve trunk producing a localized muscle contraction. The further the fibres grew from the implant, the fewer end-plates were formed. Many fibres seemed to end in a long, thin, varicose branch.

Implants into a normal, innervated muscle produced very few end-plates and these were possibly on muscle fibres denervated by injury during the implantation. The end of the implanted nerve developed a neuroma like that formed in fascia—numerous fine nerve fibres which appeared to end blindly without end-plate production. However, where the nerves came in contact with muscle fibres, the nuclei of the latter appear to orientate themselves along the nerve.

Experiments on hair growth in the rat. By A. Durward and K. M. Rudall. *Department of Anatomy, School of Medicine, Leeds* 2.

In the rat, hair grows continuously but not all over the body at the same time. The pattern of growth takes the form of a wave commencing ventrally and proceeding to the dorsum. The passage of such a wave, which may be up to $\frac{1}{2}$ in. wide, takes approximately 6 weeks.

Histologically, in the region of the wave of active growth, it is found that all the hairs grow simultaneously, their roots extending downwards into the hypodermis and commonly being deflected on reaching the panniculus carnosus. The growing hair roots are accommodated in an increased hypodermic layer of fat, which is of appreciable thickness and sharply confined to the zone of hair growth. Injection experiments indicate that accompanying the wave of growth there is a very considerable increase in the vascularity in the region.

Experiments have been carried out in an attempt to elicit what factors may play a part in determining the particular pattern of hair growth seen in the rat. Section of segmental nerves to the trunk with resulting denervation of one side of the animal induces no modification in the ventrodorsal pattern of growth wave. In other experiments a zone of depilation was induced by means of X-rays. The subsequent study of the animal showed that the wave of growth proceeding from the venter reached the edge of the bald zone but recommenced dorsal to the zone of depilation and proceeded to the mid-dorsal line, which it reached simultaneously with the wave ascending on the opposite side. A comparable experiment in which a strip of skin was removed along the side of the animal again showed that the wave of growth proceeded from the venter towards the dorsum, but halted at the line of the incision. Later it appeared dorsal to the incision, and reached the mid-line at the same time as the wave from the opposite side. These two experiments suggest that the wave does not proceed into an area of skin which has not completed its normal phase of quiescence.

Further experiments involved the rotation of skin areas on the side of the animal. The subsequent study of the growth waves indicated clearly that the wave of growth was rotated with the skin.

It would appear that the pattern of hair growth is independent of the innervation of the skin and that the wave is an inherent property of the skin.

A pre-somite human conceptus of about 14 days of age, with special reference to the yolk-sac formation. By W. R. M. Morton. *Department of Anatomy, Queen's University, Belfast*

A complete human conceptus recovered from curettage material, and in a fair state of histological preservation, is described. The embryonic shield, which has been sectioned slightly obliquely to the sagittal and dorsi-ventral planes, shows a small primitive streak. The amniotic cavity is smaller than the yolk-sac, and does not show an amniotic duct. The yolk-sac is prolonged distally by a very fine duct, which connects the proximal part with a distal expansion of larger size. The cells lining the proximal part and the duct are cuboidal endodermal cells, but those lining the distal part are flattened, and in places appear to be absent, the distal wall then being formed by flattened mesodermal cells only. The distal expansion approaches one side of the chorionic vesicle and is connected by its covering mesoderm to the chorionic mesoderm. Its size and general appearance suggests that it may be in process of being nipped off from the exocoelomic or primary yolk-sac cavity. Vasculogenic spaces are present in the mesoderm over the proximal part of the yolk-sac. The clinical data suggest that ovulation occurred in mid-cycle (21 days), and that the conceptus is 14 to 15 days old. This would agree with the general stage of development of the conceptus.

FEBRUARY 1949

An ordinary meeting of the Society, for the Session 1948–49, was held on Friday, 18 February 1949, in the Department of Anatomy, London Hospital Medical College, Turner Street, London, the President (Prof. A. B. Appleton) in the Chair.

The following are authors' abstracts of the papers read:

Afferent nerve supply of innominate-subclavian arch in the human embryo and foetus. By J. D. Boyd. *Department of Anatomy, London Hospital Medical College, Turner Street, London, E.* 1

The pressor-receptor nerve supply to the right fourth branchial arch artery in most mammals is situated on the commencement of the subclavian artery, immediately beyond the bifurcation of the innominate artery. The development and distribution of this portion of the pressor-receptor system has been studied on silver-impregnated human embryos and foetuses and the investigation demonstrates that, in man, the principal receptor region is in the wall of the innominate artery. The endings of the receptor fibres are located chiefly on the postero-lateral aspect of the vessel and are distributed throughout the whole length of the vessel, but are richest in number near the bifurcation. Details of the origin of the pressor-receptor fibres from the related nerve trunks are described.

Regenerated sensory pattern in grafted skin. By J. Hutchison and G. M. Wyburn. *Department of Anatomy, The University Glasgow,* 2

The sensory pattern in grafted skin was tested in a series of cases composed mainly of skin grafts to face and fingers. In the successful graft, after a suitable lapse of time, it was found that the grafted skin assumes the sensory pattern of the recipient area.

The method of comparative assessment of the sensory pattern is described and the significance of the results discussed.

The innervation of the human quiescent nipple. By E. P. Cathcart, F. W. Gairns and H. S. D. Garven. *Department of Anatomy, The University, Glasgow,* 2

In eighteen cases, ages 27–60 years, the human nipple in the female has been examined by the Bielschowsky-Gros method. An extremely profuse sensory innervation has been found. This is located largely in and near the sphincter region of the duct mouths. Sensory endings of very varied type have been demonstrated. The skin surface innervation over the nipple and areola was found not to be similar to other typical skin areas. A rich sympathetic ground plexus has been shown in varying appearances at varying depths of impregnation.

On the pre-natal growth of the mammary gland rudiment. By B. I. Balinsky. *Institute of Animal Genetics, King's Buildings, West Mains Road, Edinburgh* 9

The intra-uterine development of the mammary gland rudiments in the mouse was studied by preserving and sectioning embryos of known age. The rate of growth of the mammary gland rudiments was determined by calculating the mitotic index and by measuring the surface of each section drawn with the aid of a camera lucida. The rate of growth of the mammary gland was compared with the growth of the whole embryo and with the growth of other embryonic tissues.

The development of the mammary gland rudiments goes through several distinct phases. The earliest rudiments, in the form of localized epidermal thickenings, are formed during the twelfth day of development. Contrary to the assertion of Turner and Gomez the mammary line is scarcely perceptible in the mouse, each gland making its appearance independently of the others. The lentoid thickening is soon transformed into a spherical body which slowly increases in size but does not change its form until the sixteenth day of development, after which the primary sprout and the secondary sprouts are formed in quick succession. The mitotic counts and volumetric determinations show that as soon as a mammary gland

rudiment is formed, its growth sinks considerably below that of the adjoining tissues and of the embryo as a whole. The gland comes into a peculiar state of diapause. This phase of inhibited growth accompanied by an absence of formative activity continues for about 5 days, until the sprouting of the gland begins. The sprouting of the gland is associated with its intensive growth, which now surpasses that of the body as a whole. There are indications that a diapause in the early (pre-natal) development of the mammary glands may be of widespread occurrence in the mammals.

Multiple ovarian grafts. By MADGE M. BREWARD and S. ZUCKERMAN. *Department of Anatomy, The Medical School, Hospitals Centre, Birmingham* 15

Compensatory hypertrophy, which is a function of the trophic influence of the adeno-hypophysis, has been mostly studied in experiments in which the body has been partly deprived of one of the organs which exhibit the phenomenon (e.g. ovaries, adrenal cortex). The opposite and complementary condition of implanting into the body more than the normal amount of a given tissue has been little, and never quantitatively, investigated. The main conclusion that has emerged from work of this kind on the ovary is that additional ovarian tissue does not 'rake' as well in normal as in spayed animals.

In the present experiments two or six additional ovaries were implanted deep to the sterno-hyoid muscles of normal mature female albino rats. All but one of the grafts in the six animals receiving two additional ovaries degenerated. The results were better in the six into which six additional ovaries were implanted. All had ova in at least one graft, four had ova in two grafts. Normal and cystic follicles were also present. Degenerated follicles were a constant feature, as was also absence of the germinal epithelium.

Quantitative study suggests that the host-animals' own ovaries did not change in weight, but that the number of follicles they contained decreased and that the rate of atresia increased. Weight changes also occur in the pituitaries and adrenals.

Experiments on the longitudinal growth of tendons in rabbits. By G. N. C. CRAWFORD. *Department of Human Anatomy, University of Oxford*

Tendons of various long flexor and extensor muscles of the limbs of month-old rabbits were exposed at operation, small indian ink marks made at intervals along the length of the tendons, and the distance between them measured. The rabbits were allowed to survive for 2–4 months after operation. The indian ink marks remained, and by again measuring the distance between them the amount of longitudinal growth which had occurred could be determined. Control experiments have been performed on young and adult rabbits in order to exclude the possibility of any abnormal effects resulting from the operative interference.

These preliminary experiments indicate that, despite many statements to the contrary, interstitial longitudinal growth occurs throughout the length of the tendon in young rabbits, but that maximal growth appears to occur near the muscle tendon junction.

Action of posterior crico-arytenoid muscle of the larynx. By V. E. NEGUS. 149, *Harley Street, W.* 1

This muscle is usually described as having its main effect on the muscular process of the arytenoid cartilage, with external rotation of the vocal process. It is to be observed, however, that lateral fibres of the muscle have the function of sliding the arytenoid cartilage outwards on its sloping facet, and thus causing the glottis to open in a triangular manner. The function of the muscle in bracing back the aryepiglottic fold is also of importance. In phonation the posticus muscle acts as an opposer to the sphincteric group in preventing excessive forward movement of the arytenoid.

The nutrient canal in the long bones of the limbs. By H. HUGHES. *Department of Anatomy, University College, Newport Road, Cardiff*

Examination of specimens in a representative collection of skeletal material showed that the direction of the nutrient canal is very variable in certain bones. A graphical analysis of this material demonstrated that the older attempts to account for such variability are not altogether satisfactory, and suggested a new approach to the problem.

An experimental study of fracture repair in *Lacerta vivipara*. By J. J. PRITCHARD and A. J. RUZICKA. *Department of Anatomy, St Mary's Hospital Medical School, Paddington, London, W. 2*

Closed fractures of the femur were produced manually and the course of repair followed histologically for 50 days.

The rate of healing was greatly influenced by the environmental temperature. At room temperature (15° C.) some osteoblastic activity was present after 40 days, but there was no new bone or cartilage formed. At 37° C. repair was greatly accelerated, but was still not as fast as in the rat.

The characteristics of the repair process may be summarized as follows:

(1) At an early stage there was active proliferation of subperiosteal cells near the fracture. A little later some proliferation of cells lining the marrow cavity was seen.

(2) There followed the deposition of a predominantly cartilaginous matrix under the periosteum and extending into the fracture haematoma. A very small amount of bone matrix was laid down at this stage in the marrow cavity and immediately adjacent to the fibrous periosteum.

(3) Union occurred by the fusion of the cartilaginous masses arising from the two fragments.

(4) The cartilage was then replaced by bone in two ways: (*a*) by osteoblastic activity after preliminary invasion by blood vessels and chondroclasts as in normal endochondral bone formation; (*b*) by direct transformation of the cartilage matrix into bone.

These results show the following distinguishing features when compared with the evidence obtained from similar studies on mammalian long bones:

(1) Slower callus formation even at comparable temperatures.

(2) The predominance of cartilage in the provisional callus.

(3) The direct transformation of cartilage into bone.

The relationships between the various cellular elements and intercellular matrices present in the callus are particularly well defined in the animal studied.

Structure of axones within brain and cord. By M. A. MACCONAILL. *Department of Anatomy, University College, Cork*

It was shown in an earlier paper (*Proc. Anat. Soc.* Nov. 1947) that the axones of cerebrospinal nerves consist essentially of the central axis-cylinder (fuchsinophobe) and an outer axolemma (fuchsinophil). 'Free' myelin is contained within the cavity formed by the splitting of the axolemma; this cavity (*myelotheca*) has a complicated form in nerve trunks but a simple form in the brain and cord; it is absent in the intra-spinal and intra-cerebral nerves in the newborn.

Further study, using an improved form of the original stain (see below), have shown that the infantile form is retained by that part of the axone which is approaching its synaptic termination. Furthermore, the actual termination (*bouton terminaux*) is wholly composed of the axolemmal substance; and the same appears to be true for the terminal stage of the axone proper.

The staining technique is as follows: formalin-fixed material is embedded in paraffin and sectioned (8μ or more). It is stained first in a mixture of lead nitrate and acid fuchsin and acid solutions, then in a mixture of haemotoxylin and acetic acid, lastly in a mixture of ammonium molybdate and liquid ammonium acetate. This technique stains the finest fibres and *boutons terminaux* red with the greatest regularity. It is a useful and rapid (15 min.) substitute for the Weigert-Pal method in class work. Full details of the method will be provided at the demonstration.

The innervation of vibrissa-like hairs in the rabbit's leg. By G. WEDDELL. *Department of Human Anatomy, University of Oxford*

The innervation of the vibrissa-like hairs following methylene blue staining is described. Briefly, from 4 to 14 myelinated nerve fibres approach the hairs from the cutaneous plexus. Close to the hair sheath at its root numerous collaterals are given off, which rapidly lose

their myelin sheaths and end in one of two ways. One group of terminals encircles the base of the hair sheath and gives rise to a basket-like network of intertwining beaded fibres. The other group gives rise to a series of non-beaded terminals, which run vertically up the sheath of the hair shaft from its base for a short distance before terminating.

It is possible to trace the nerve fibres from the hairs into the cutaneous nerve plexus for some distance, but, owing to the large amount of collateralization, it is not possible to determine how many nerve fibres within the nerve trunk supplying the area of skin in which the hairs are situated are associated with a single hair. Degeneration experiments indicate that at least two nerve fibres are involved.

The histological findings described above have been correlated with the action potentials led off from the nerve supplying the area of skin in which the hairs are situated. By this means it has been possible to determine the number of nerve fibres associated with a single hair in the autonomous zone of the nerve.

An experimental study of optic pathways in *Lacerta vivipara*. By J. A. Armstrong. *Department of Anatomy, St Mary's Hospital Medical School, Paddington, London, W. 2.*

In a number of specimens of *Lacerta vivipara* the left eye was removed under ether anaesthesia, and the animals allowed to survive from 6 to 36 days. They were kept in heated cages at approximately 30° C., since it was found that at room temperature (about 15° C.) degenerative changes were very slow.

The brains were impregnated by the Nonidez modification of Cajal's technique, and serial sections were studied for axonal and terminal degeneration. Degenerative changes showed a sequence similar to that described in mammals, but the process was much slower.

Complete decussation of degenerating optic fibres was observed, their course in the main (or marginal) optic tract was traced to the tectum of the mid-brain, and their differentiation from the extensive supraoptic system clearly demonstrated. The basal optic root (posterior accessory optic tract) is shown to be predominantly and probably entirely retinal in origin.

Terminal degeneration is shown early by the appearance of large numbers of annular and bulb-like end feet, and of fine terminal fibres showing fragmentation and swelling. Such appearances were seen in the lateral geniculate nucleus (dorsal and ventral parts), the pretectal region, the tectum, and the nucleus of the basal optic root. End feet were only rarely seen in these regions of the normal brain.

The nerve supply of the kidney. By G. A. G. Mitchell. *Department of Anatomy, Victoria University, Manchester* 13

The renal nerves are derived from the coeliac plexus, the thoracic and lumbar splanchnic nerves, the upper and lower ends of the intermesenteric nerves, and sometimes from the superior hypogastric plexus.

The renal branches from the coeliac and aorticorenal ganglia contain sympathetic and probably vagal fibres.

The greater splanchnic nerve occasionally, and the lesser splanchnic nerve almost invariably, send direct filaments to the aorticorenal ganglion or renal plexus, while the lowest thoracic and upper lumber splanchnic nerves end in the posterior part of the renal plexus. There is a quantitative inter-relationship between the sizes of the lower and lowest thoracic and upper lumbar splanchnic nerves.

The upper renal branches from the intermesenteric nerves run almost directly to the renal hilum. Interesting renal nerves arise from the lower ends of the intermesenteric nerves or from the superior hypogastric plexus. Except near their terminations they are separate from the other renal nerves, they often show macroscopic or microscopic collections of ganglion cells along their course, and on anatomical and embryological grounds it is suggested that the ureter, pelvis and renal collecting tubules may receive their parasympathetic supply via these nerves from the caudal rather than the cranial outflow.

Renal vascular anomalies are common and there is some evidence that cranial or caudal

nerve-shifts are associated with vascular variations, just as pre- or post-fixation elsewhere may accompany skeletal variations.

The nerves mentioned unite in a plexiform manner around the renal artery and this plexus divides into subsidiary plexuses which accompany the arterial branches into the kidney. Ganglia of varying size occur in the plexus, but none were discovered on the intrinsic renal nerves.

E. Ullmann, L. E. McGee and K. J. Franklin (Physiology Department, St Bartholomew's Hospital Medical School) exhibited a film showing 'Anosic diversion of the renal cortical blood flow'.

APRIL 1949

An ordinary meeting of the Society, for the Session 1948–49, was held on Friday, 22 April 1949, in the Royal Veterinary College, Royal College Street, London, the President (Prof. A. B. Appleton) in the Chair.

The following are authors' abstracts of the papers read:

Implantation and the yolk-sac placenta in the cat. By E. C. Amoroso and C. B. Murray. *Department of Anatomy, Royal Veterinary College, London, N.W.* 1

In the cat, implantation is of the central type with the embryonic disc oriented toward the antimesometral pole of the uterus. At first the blastocyst is spherical and the trophoblast is equally thick and well developed on the whole surface of the sphere. However, as development proceeds the trophoblast at the poles lags behind the invasion of trophoblast in the central zone, and by the fourteenth day the invasive differentiation of the trophoblast is reduced to an annular band which encircles the equatorial portion of the vesicle. In the polar regions there is never any penetration of the trophoblast, the relationship between it and the uterine epithelium remaining one of simple contact (placenta epitheliochorialis). The embryonic disc remains directly exposed to the uterine lumen until the sixteenth or seventeenth day, when the chorio-amniotic folds unite to form true chorion, which then becomes applied to the antimesometrial wall of the uterine swelling, thus completing the area of attachment.

In the cat the yolk-sac is completed while apposition between ovum and endometrium is being effected, and as it enlarges the progressive invagination of its roof by the embryo results in the so-called 'incomplete inversion' of the sac. Three functional subdivisions of the yolk-sac placenta are established during, or shortly after, implantation. These are: (1) the non-vascular bilaminar omphalopleure; (2) the non-vascular trilaminar omphalopleure; and (3) the vascular trilaminar omphalopleure (chorio-vitelline placenta). The bilaminar omphalopleure is completed before implanation. Its area is reduced when the growth of the mesoderm between the endoderm and the trophoblast transforms it into the trilaminar omphalopleure, also non-vascular. The chorio-vitelline placenta comes into existence when the trilaminar omphalopleure, vascularized by vitelline vessels, enters into placental relationships with the maternal tissues. It begins to form before the bilaminar omphalopleure has been completely invaded by mesoblast and in addition to its haemopoietic function accumulates a fluid of high nutritional value for the foetus. It disappears between the twenty-first and twenty-fourth day when the vascular splanchnopleure separates from the non-vascular true chorion.

In its histological structure the chorio-vitelline placenta consists of tongue-shaped lamellae of trophoblast which are, at first, feebly excavated by foetal mesenchyme and which penetrate into the uterine mucosa to variable distances. The mesodermal cores are, however, completely devoid of a foetal circulation. The trophoblast occurs to some extent as syncytiotrophoblast, but in the main it is formed by cytotrophoblast contiguous to the chorionic mesenchyme. The maternal vessels in the immediate pathway of the approaching trophoblast are dilated and possess enlarged endothelial cells surrounded by delicate wisps of reticular fibres. These vessels withstand successfully the process of invasion and erosion, though occasionally local blood extravasations are encountered. This mode of invasion of

the endometrium by the trophoblast results eventually in the establishment of an endotheliochorial placenta. Nevertheless, it is quite clear that in the placental area the only region of intimate association of the foetal and maternal circulations is in the neighbourhood of the maternal vessels, which are located at the base of the lamellae (i.e. on the placenta face) and which are separated from the area vasculosa by a thin layer of trophoblast.

The pronephros and mesonephros of the duck. By J. DAVIES. *School of Anatomy, University of Cambridge*

There is much confusion in the literature as to what constitutes the pronephros of the avian embryo. Balfour (1885) was at fault in mistaking the coelomic funnels of the developing Müllerian duct for the pronephros. Sedgwick (1880) previously described the well-developed external glomerulus of the chick and observed that it lay in the region of the mesonephros. He also showed that the external glomerulus was continuous caudally with a series of transitional glomeruli which were only partially shut off from the coelomic cavity. A similar external glomerulus and transition zone was described by Wiedersheim (1890) in the crocodile and turtle and was interpreted by him as a gradual transition from pronephros to mesonephros. The development of the kidney has been studied in the duck and compared with that of the crocodile and turtle. The interpretations of the above authors are not confirmed. It is suggested that the processes are determined by the developmental pattern of the angioblastic tissue in the nephrogenic mesoderm.

Müller's (spheno-maxillary) muscle in the human embryo and foetus. By J. D. BOYD. *Department of Anatomy, London Hospital Medical College, Turner Street, London, E.* 1

That portion of the unstriated musculature of the orbit, first described by Müller, which lies in the region of the inferior orbital fissure, was investigated in a large number of human embryos and foetuses. The mesenchymatous condensation from which the muscle is derived can be identified at the 14 mm. stage and myoblasts are recognizable at the 20 mm. stage. Well-differentiated unstriped muscle is present at the 47 mm. stage. At the 150 mm. stage the muscle is very extensive and possesses a bulk at least equivalent to that of the inferior oblique muscle. The attachments of the muscle and its relationships, nerve supply and possible developmental origin are discussed.

Growth changes in the mental nerve and their relationship to the direction of the mental foramen. By R. WARWICK. *Department of Anatomy, The University, Manchester* 13

In the great majority of adult human mandibles the mental foramen is directed upwards and backwards relative to the alveolar plane, whereas the direction is forwards in late foetal life and forwards and slightly upwards in early post-natal life.

Dissection indicates that the mental nerve itself is the largest element of the mental neurovascular bundle, and that the first part of its course coincides with the grooving of the foramen. In foetuses the nerve passes towards the angle of the mouth in a forward and slightly upward direction; in adults its course is more vertical, but the nerve is usually tortuous.

In view of the major distribution of the mental nerve towards the angle of the mouth, this point was adopted as a landmark on the course of the nerve in the living. Such a procedure is necessary because of the practical impossibility of obtaining children or adolescents for dissection. To obtain information about the course of the nerve at different ages, recourse was made to teleradiography. The angle of the mouth was marked and the course of the nerve determined by joining this point to the mental foramen.

It will be demonstrated that the angle subtended between the nerve and the alveolar plane varies with age, and that this influences the appearance of the mental foramen.

Osteological racial differences in the newborn. By W. C. Osman Hill. *Department of Anatomy, University New Buildings, Teviot Place, Edinburgh* 10

On the basis of von Baer's principle, it is usually tacitly assumed that racial differentiation has not commenced at full term or at most affects certain external features. Statements in the literature relating to neonatal cranial differences are contradictory; Aeby, for example, advocating the view that in the neonatus of all races the skull is of the same shape, whilst Abbie (1947) concludes that the cranial index of all human newborns ranges around 78–82, a category which he terms paedocephalic. On the other hand, Vogt and others consider that osteological differences already exist at birth, especially between negro and European crania, whilst Hauschild has even enumerated differences in the chondrocranium.

In a recent investigation of the skeleton of a neonatal Veddah compared with Sinhalese and European skeletons of similar development, a number of differences were revealed. Though Europeans and Sinhalese fall into Abbie's paedocephalic group, the Veddah does not. Prognathism is present at birth in Veddah and Hottentot and has previously been reported in the negro. Distinctions in individual cranial bones (parietals, exoccipitals, vomer, etc.) are in accord with these deductions. Small size of the sella turcica in the Veddah newborn is significant in connexion with Keith's teachings on endocrine factors in racial differentiation. Other racial characters in the Veddah are precocious calcification of teeth, frequency of segmental variations in the axial skeleton and peculiarities in scapula, ilium and limb proportions.

Extension at the metacarpo-phalangeal and interphalangeal joints of the thumb. By H. Harris and J. Joseph. *Department of Anatomy, University College, Gower Street, London, W.C.* 1

It was observed that there was a marked difference in the degree of extension at the above two joints in the two investigations, and it was thought that a study of the variation of this movement in a random sample would be of interest. A sample consisting of 133 males and 100 females was X-rayed and the angles at the joints were measured. A very wide variation in the degree of extension was found. A considerable number of subjects showed increased extension at the metacarpo-phalangeal joint when the metacarpal was flexed and abducted at the carpometacarpal joint. This was also very variable in extent, but when marked it amounted to what was almost a dislocation at the metacarpo-phalangeal joint. Females showed this more than males, and in both sexes it was more marked in the left than in the right hand. A group of male Indians was studied and they showed a significant increase in the amount of extension at the interphalangeal joint. They also showed an increase in the number of 'hyper-extensors' at the metacarpo-phalangeal joint. A similar group of Africans is being investigated.

The shape of the metacarpo-phalangeal joint was found to be very variable. When the joint surfaces were flat, hyper-extension did not appear to take place. Similarly, individuals with this type of joint showed limited flexion.

The incidence and appearance of the sesamoid bone at the interphalangeal joint of the thumb is being investigated. It has been found that there is no relation between its presence and the amount of extension at the interphalangeal joint.

An investigation of the distribution of medullated fibres in the vagus nerve of the rabbit. By D. H. L. Evans. *Department of Anatomy, University College, Gower Street, London, W.C.* 1

The calibre spectrum of the medullated fibres of the vagus nerve and its branches in the neck, thorax and abdomen has been investigated. As the nerve descends in the thorax there is a progressive decrease in the number of medullated fibres as these are distributed in the recurrent laryngeal, cardiac, bronchial and oesophageal branches. The vagus in the abdomen therefore consists almost entirely of non-medullated fibres. The recurrent laryngeal nerve near its origin from the vagus contains two groups of fibres, large and small in diameter. The small fibres are distributed to the oesophagus and trachea, so that the nerve at its entry into the larynx is composed almost entirely of the large fibres.

The number and distribution of sensory fibres having their parent cells in the ganglion nodosum was investigated by cutting the nerve trunk above this ganglion and determining the medullated fibre distribution in the nerve and its branches three weeks from the time of section. This resulted in approximately a 50% reduction in the number of fibres in the cervical region.

All the small fibres in the recurrent laryngeal survived and are therefore regarded as sensory to the trachea and oesophagus. All the large fibres which are motor to the laryngeal musculature degenerated. An estimation of the proportion of sensory fibres in the thoracic part of the vagus proved more difficult because of communications with the nerve of the opposite side.

The anatomy or popliteal thrombosis. By F. R. WILDE. *Department of Anatomy, The University Manchester* 13

Spontaneous thrombosis of the popliteal artery may be shown by arteriographic studies to occur at the level of the knee-joint line.

The present investigation was undertaken to ascertain whether any anatomical feature might explain this precise localization.

Fresh knee-joints, both foetal and adult, were examined as frozen sections, and by dissection. The popliteal artery was found to be bound to the capsule of the knee-joint by the deep fascial sheath between the two heads of the gastrocnemius muscle.

Spontaneous popliteal thrombosis is usually a complication of peripheral vascular disease. In such cases fibrosis extending into this fascial sling binds the artery firmly to the capsule of the knee-joint, and abolishes the normal smooth contour of the vessel when the knee is flexed.

Spontaneous thrombosis may occur however in a normal artery, and here again it is suggested that the fascial sling from gastrocnemius accounts for the site of onset of thrombosis.

JULY 1949

The Summer Meeting of the Society, for the Session 1948–49, was held in the Bute Department of Anatomy, University of St Andrews, on Friday and Saturday, 1 and 2 July 1949, the President (Prof. A. B. APPLETON) in the Chair.

The following are authors' abstracts of the papers read:

The reaction of the intervertebral disc to experimental lesions. By R. WALMSLEY and J. W. SMITH. *Bute Department of Anatomy, The University, St Andrews, Fife*

This is a preliminary report on the changes that occur in the intervertebral discs of rabbits after incision of the annulus fibrosus and loss of the nucleus pulposus. The mechanism of the vertebral column and the form of the vertebral epiphyses differ so greatly in pronograde and orthograde animals that the results that have been observed in rabbits would not warrant the assumption of similar changes in man. The reactions of the various tissues would, however, appear to be of interest.

The ventral aspects of the intervertebral discs have been incised in thirty-seven rabbits through a transperitoneal approach and the nucleus pulposus allowed to escape; the animals have been sacrificed at periods varying from 2 days to 21 months. After incision the superficial part of the annulus heals rapidly but the deeper part of the wound persists and is recognizable even after one year. The cells in the retained part of the nucleus pulposus proliferate and the central part of the disc is thereafter occupied by fibro-cartilage; a protrusion from this tissue extends ventrally and lies in the deep part of the wound, which it finally obliterates.

Cartilage cells are increasingly apparent in the anterior part of the annulus during the second and third months, and by the end of the third month this commences to ossify. The growth of bone is progressive throughout the period over which observations have been made and a bony ankylosis is established between the contiguous ventral borders of the vertebrae.

Limitation of movement at the subtalar and calcaneo-cuboid joints. By J. W. Smith. *Bute Department of Anatomy, The University, St Andrews, Fife*

At the hip and knee joints the movement of extension is finally arrested by a mechanism which is dependent on three factors; the shape of the articular surfaces, the disposition of the joint ligaments and, usually, the force of body weight.

The present investigation was carried out on adult material to ascertain if a similar mechanism exists at the subtalar and calcaneo-cuboid joints.

As a result of the examination of the curvature of the lines of movement, and the disposition of the ligaments, it seems that at both these joints the necessary factors are present in most cases to arrest movement in full pronation. It is suggested that this mechanism may be of importance in maintaining the arched form of the weight-bearing foot.

Some observations on the human foetal ovary. By P. Bacsich. *Department of Anatomy, The University, Glasgow* 2

Ovaries from twenty human foetuses ranging from 7 months to full term were examined in serial sections. Almost complete absence of multinuclear ova was observed. Results seem to support the view expressed in earlier communications (Bacsich, 1946, 1949), that the abnormal ova of the neonatal ovary represent gonadotropic hormone withdrawal phenomena.

Some observations on the histology of the infant testicle. By R. Mitchell. 971, *Sauchiehall Street, Glasgow, C.* 8

Testicles of twenty infants from birth up to 1 year were examined histologically.

After slight initial rise there was a gradual reduction in the diameter of the seminiferous tubules. The number of spermatogonia exhibited similar behaviour. Abnormal, multinuclear spermatogonia were frequent in the first few weeks after birth, later their number diminished. It is believed that these changes are comparable to those found in the neonatal ovary (Bacsich, 1946, 1949), and may also be due to the sudden withdrawal at birth of the maternal gonadotropic hormonal influences.

A note on pigmentation of the skin. By J. Kirk. *Department of Anatomy, Middlesex Hospital Medical School, London, W.* 1

A series of histological sections of skin will be shown, demonstrating the arrangement of pigment in the cutaneous tissues of the following specimens:

(1) normal European; (2) Negro; (3) Benign and malignant melanoma; (4) Addison's Disease; (5) Blood pigmentation; (6) Tattooed skin.

Electromyographic investigation of muscle movement patterns studied with surface electrodes on the anterior abdominal wall in man. By W. F. Floyd and P. H. S. Silver. *Department of Anatomy, Middlesex Hospital Medical School, London, W.* 1

Surface electrodes were placed over the muscles on both sides in pairs as follows: over the rectus, in the loin over the external oblique, and on the internal oblique over the aponeurosis of the external oblique, but lateral to the rectus. The muscle action potentials picked up by the six pairs of electrodes were fed through a 6-channel amplifier with ink writing oscillographs (Ediswan electro-encephalograph) and recorded simultaneously. In this way the pattern of muscle actions was demonstrated.

In the recumbent position complete relaxation of the whole abdominal wall is usual, and no action potentials are seen. In the erect posture the rectus remains relaxed; the external oblique shows slight activity but can be voluntarily completely relaxed; the postaponeurotic fibres of the internal oblique (which are the only ones studied) are always in a state of tonic contraction over the inguinal canal.

The action of these three muscles as fixators can be demonstrated in the recumbent position. When the head is raised against gravity, it is mainly the rectus that comes into

action as a fixator of the thorax; the obliques contract to a less extent. When the legs are raised off the couch, all three groups of muscles are used approximately equally as fixators of the pelvis.

All movements involving active expiration, such as talking, singing, coughing, and expiration against resistance, e.g. in straining, employ the same movement pattern on repetition in the same people and are similar in different normal subjects. The action is predominantly in the internal and external oblique muscles. The rectus comes into action later and may not be involved until a maximal effort is made: the degree to which it is involved varies in different people, but the general picture is similar.

Movements of digits in ulnar paralysis. (Film.) By R. Bowden, J. Napier and J. Whillis. *Department of Anatomy, Royal Free Hospital Medical School, 8, Hunter Street, London, W.C. 1*

The film demonstrates first the instability of the extensor communis tendons over the knuckles in a case of ulnar paralysis where the interossei, which normally hold the tendon in a central position, are paralysed. The patient is a child and instability of minor extent is shown in the normal hand. The lack of stability of the ring finger in the 'upstroke of drawing' movement is demonstrated in ulnar palsy in an adult. This is due to weakness of the intrinsic muscles, particularly of the lumbrical. Instability of the extensor communis is not so obvious in the adult. The normal excursion of the extensor communis and its stability in all movements is shown in the final part of the film.

The renal fascia. By G. A. G. Mitchell. *Department of Anatomy, The University, Manchester 13*

The anterior and posterior layers of renal fasia are usually said to fuse only above and laterally, but they are also united below and medially. The suprarenal lies within the perirenal sheath, being partially separated from the kidney by a thin fascial lamina. Above, the two layers are firmly fused to each other and to the diaphragmatic fascia. Laterally they are also united and while this union is generally firm, it is not invariably so. Below, the layers are weakly united, except around the ureter, where they fuse with the periureteric tissue. Medially both layers merge into the mass of connective tissue surrounding the great vessels, and normally this presents a barrier to the medial spread of effusions within the perirenal space. This mass of connective tissue is particularly evident in the region of the coeliac axis, superior mesenteric artery and coeliac plexus, and it is less definite below the level of renal hilum. The structures entering and leaving the kidney penetrate this connective tissue and are closely invested by it. Each posterior layer of renal fascia is partially blended with the fascia covering the corresponding psoas and diaphragmatic crus, and through this connexion both layers obtain an indirect attachment to the intervertebral discs and anterior spinal ligaments. Distinct layers of fascia either anterior or posterior to the great vessels can be created only by artificial dissection of the connective tissue surrounding them.

Injection experiments reveal that the fusion between the layers is weakest below in the region of the ureter, and that on the medial side it is firmest above and gradually weakens from above downwards. In the hilar region the peritoneum is more closely attached to the anterior layer of renal fascia than elsewhere, and when rupture occurs here the peritoneal cavity is usually invaded.

The return of sweating in grafted skin. By I. McGregor. *Department of Anatomy, The University, Glasgow*

The object of this investigation was to obtain information on the return of sweating to grafted skin. The work is confined to full thickness skin grafts to the palmar aspect of fingers.

The number of sweat glands of finger skin is normally very much greater than of the corresponding area of abdominal skin and thus there was an opportunity of finding out if there was a formation of sweat glands in grafted skin.

The method of investigation is described, and from the results it is concluded: (1) that

regeneration of sweat-gland activity does occur; (2) no development of new sweat glands takes place in the grafted skin; (3) the pattern of spatial distribution of sweat glands remains similar to that of the donor skin; (4) the grafted skin acquires the physiological sweat pattern of the recipient area, e.g. skin grafts on the fingers acquire the characteristics of emotional type of sweating.

The anatomy of jaw movements in foetal sheep. By J. H. Scott.
Department of Anatomy, Queen's University, Belfast

The late Sir Joseph Barcroft and his co-workers found that stimulation of foetal sheep produced rhythmic movements, which included opening of the mouth, at about the 42nd day of gestation.

Serial sections of foetal sheep show that the joint between the malleus (with which Meckel's cartilage is continuous) and the incus commences to develop at about the 40th day. The temporo-mandibular joint, however, does not begin to appear until about the 48th day.

From the 48th day until about the 60th day movements of the lower jaw involve the temporo-mandibular joint and the joint between malleus and incus, as Meckel's cartilage is continuous with the malleus until about the 60th day of gestation. At this time also calcification commences in the crowns of the developing teeth.

An histological and sensory investigation to determine the role of the articular capsule in joint sensation. By E. P. Samuel. *Department of Anatomy, The University, Manchester* 13

A study of twenty normal human knee joints revealed that the nerves accompanying the blood vessels in the articular capsule migrate from one to another, forming a coarse network of nerve bundles in the capsular ligament and a more delicate network of individual fibres in the synovial membrane. From the fibres of this network, from the filaments associated with the blood vessels and from the nerve bundles which enter the joint independently of the blood vessels, a fine plexus of non-medullated fibres is derived. The nerves associated with blood vessels supply them, and individual fibres are observed to terminate in simple and complex endings.

Experiments on six human volunteers, carried out in co-operation with Dr J. H. Kellgren, demonstrated that the fibrous capsular ligament is pain and pressure sensitive. Observations on the knee joints of five patients and of one trained human volunteer showed that the synovial membrane is only slightly sensitive to pain and does not give rise to pressure sensations. It appears that almost all the sensory impressions from the articular capsule arise in the fibrous capsular ligament.

Though it is not possible to correlate exactly these observations with particular nerves in the articular capsule, the findings suggest that the difference in sensitivity of the capsular ligament and synovial membrane is to some extent an expression of the difference in the density of their nerve supply.

Alkaline phosphatase in the mesonephric and metanephric tubules of man. By J. S. Baxter. *Department of Anatomy, The University, Bristol* 8

The metanephros has been examined for cytoplasmic alkaline phosphatase in a series of human foetuses from 80 mm. C.R. length until term and in several babies up to 12 days of age. The enzyme has been found in the cells of proximal convoluted tubules from a certain stage of structural differentiation onward. The enzyme was not present in developing tubules prior to this stage, being present only when the cell cytoplasm next the tubule lumen had become modified to become a brush border. This structural alteration in the cell cytoplasm caused a tinctorial differentiation between it and the remainder when certain trichrome stains were used.

Two embryos have also been studied, one being 20 mm. and the other 26 mm. C.R. length. In each, cytoplasmic alkaline phosphatase was found in the proximal 'secretory' segment of *intact* mesonephric tubules.

Some points arising out of the results will be discussed.

The source and composition of the uterine milk in the mare. By E. C. Amoroso.
Department of Anatomy, Royal Veterinary College, London, N.W. 1

Distributed over a part of the endometrium of pregnant mares, specialized cup-shaped structures are found which have been referred to as 'mucosal craters' or 'endometrial cups'. As early as 1912, Schauder reported their occurrence in pregnant uteri of the mare from the sixth to the twentieth week and associated their presence with the formation of uterine milk which, in his opinion, is derived in part from an early and extensive degeneration of the superficial portions of the mucosa. These early observations have been confirmed from an examination of several pregnant uteri, and additional morphological facts have been added as a result of this investigation. An attempt has also been made to collect physiological evidence based on histochemical studies, and from these preliminary studies new ideas may be formulated concerning the origin and function of these differentiations.

The structures in question are limited to the fertile horn and vary in maximum diameter from a few millimetres to about 5 cm. They occur most frequently at the junction of the body and horn of the uterus and most commonly occupy a circular or horseshoe-shaped area of the endometrium in the vicinity of the attachment of the yolk-sac. The allantochorion opposite the craters on the uterine wall is devoid of villi and the endometrium at these points also lacks crypts. There is no attachment of the membranes and the uterine epithelium is wanting; an irregular space, representing all that is left of the uterine lumen at the level of the cup, separates the trophoblast from the endometrium in the small area above the uterine depression. The only thing about this early relationship which is not typical of a syndesmochorial placenta is the absence of any genuine connexion between the foetal and maternal tissues. The space contains a granular eosinophilic coagulum which is derived from the breakdown of mucosal tissue, supplemented by the secretion of the uterine glands and of the intact portions of the uterine epithelium.

The histochemical reactions of the uterine secretions under consideration lead us to believe that they contribute to the nourishment of the implanting blastocyst and possess, in addition, an unusually high concentration of gonad-stimulating hormone. The cups seem to be composed of three types of cells: (1) numerous small round cells; (2) large polyhedral cells lying between the ducts of the uterine glands; and (3) a few multinucleate giant cells which appear to be degenerating. The interglandular cells are the conspicuous features of the cups when these are first formed and in their development they resemble most closely the decidual cells of the endometrium which are seen during pregnancy in other forms; they differ, however, from 'typical' decidual cells in that no stainable glycogen could be demonstrated, either by Best's Carmine method or by Bauer's method.

Neurological techniques in relation to the anatomy of living corneal nerves. By E. Zander and G. Weddell. *Department of Human Anatomy, University of Oxford*

A method of preparing the whole cornea from various animals for neuro-histological examination is described. It involves the use of 'Methylene Blue', which stains the living nerve axis cylinders specifically, uniformly and constantly throughout the cornea. It also involves a method of 'after treatment' that allows the cornea so stained to be excised, mounted and preserved as a preparation for examination by ordinary microscopical methods without any detectable morphological change.

Pictures of living stained and unstained corneal nerve fibres obtained with the slit-lamp microscope, and those seen under the phase-contrast microscope in slices of freshly excised cornea, have been shown to be strictly comparable with those obtained by the histological technique described. The pictures given by this Methylene Blue technique have also been compared with those obtained by the best impregnation methods, and the results are discussed. Using this technique it has been concluded that the beads on the course of some of the fine nerve fibres are not artefacts. It has also been shown that there are no specialized nerve end formations in the cornea of different vertebrate species. The presence of the so-called terminal reticulum and sympathetic ground plexus in the cornea are shown to be the result of non-specific staining.

Observations on the innervation of the cornea. By G. WEDDELL and E. ZANDER. *Department of Human Anatomy, University of Oxford*

A description is given of the innervation of the normal cornea from a number of vertebrate species.

It has been shown, among other things, that the corneal nerve axis cylinders terminate in both the substantia propria and the epithelium in the form of a series of interconnected beads. The terminal beaded fibres in the epithelium run mainly between the cell layers, and end on and between the cells but never in them.

A single non-beaded axon traced from the substantia propria through Bowman's membrane into the epithelium is found to give rise to a very large number of beaded fibres; some proceed to anastomose with one another, forming a protoplasmically continuous network from which further beaded fibres arise to terminate freely; others proceed to their terminations without forming anastomoses. Anastomoses occur only between the fibres derived from a single parent axon. The terminals in the substantia propria also take the form of a series of interconnected beads, but no anastomotic net formation is seen and the beads from a single axon are distributed in a circumscribed volume of tissue compared with wide distribution of similar beaded terminals in the epithelium.

The physiological implications of these findings are discussed.

Nerve supply of the tonsil. By J. D. BOYD. *Department of Anatomy, London Hospital Medical College, Turner Street, London, E.* 1

The nerve supply of the tonsil was investigated in de Castro, Bielschowsky, Cajal and Spielmeyer material from a number of human embryos, foetuses and post-natal stages. Tonsils from pig, dog, shrew and rabbit were also studied. In the human material the tonsil is surrounded on its non-epithelial surface by a plexus of nerve bundles derived principally from the tonsillar branches of the glossopharyngeal nerve. Scattered autonomic nerve cells are occasionally present in this plexus and there is a small ganglion in the main tonsillar nerve. Both myelinated and unmyelinated fibres are present in the plexus. From the plexus fibre bundles pass into the tonsil itself, chiefly along the fibrous trabeculae and around the blood vessels. In the tonsil the fibres terminate in three main fashions:

(1) In a plexus under the superficial epithelium. This plexus is also present around the epithelium of the crypts but is not so marked in this position. No nerve fibres were seen in the epithelium itself.

(2) In relation to the blood vessels—especially where arterioles appear to be opening directly into venules (? arterio-venous anastomoses).

(3) Nerve fibres can be traced into the follicles, where they terminate as finely branching fibrils which occasionally appear to form a network. No precise relationship of these fibrils with the cells of the follicles could be determined.

With the exception of the dog, where only Bielschowsky material was studied, similar findings are recorded for the other mammals examined.

Medullation of the optic nerve in the mouse. By G. J. ROMANES. *Department of Anatomy, University New Buildings, Teviot Place, Edinburgh* 10

Some numerical estimations of medullated nerve fibres in the developing optic nerve of the mouse are presented. Special reference is made to the variations in number along the length of the nerves and the significance of these variations to the determination of experimentally induced changes in the rate of medullation.

Preganglionic fibres of the vagus. By F. GOLDBY and A. MOHIUDDIN. *Department of Anatomy, St Mary's Hospital Medical School, Paddington, London, W.* 2

The rootlets of the right vagus have been cut intracranially in six cats. At intervals varying from 2 to 10 days after the operation, part of the thoracic vagus distal to the origin of the pulmonary and cardiac branches has been examined for evidence of fibre degeneration. A myeline sheath stain (osmium tetroxide) and an axonal stain (silver impregnation,

Romanes' method) has been used. Positive evidence of degeneration has been found by both methods, but in a surprisingly small proportion of the fibres present.

A single experiment has been performed in which the right vagus was cut in the thorax below the origin of the cardiac and pulmonary branches, in a kitten ten days old. The kitten was killed 9 days later and the medulla examined for retrograde cell changes in the vagal nuclei. Slight chromatolytic changes were observed in the dorsal nucleus of the vagus on the right side. So far as it goes, the evidence of this single experiment is consistent with the results of the other experiments.

Although the belief is generally held that preganglionic fibres of the vagus supplying abdominal viscera arise from cells in the medulla, there is very little anatomical evidence on which it can be based. From time to time alternative origins have been suggested, e.g. from cells in the nodose ganglion (Heinbecker & O'Leary, 1933, *Amer. J. Physiol.* **623**, 106). The above experiments show that at least a few such fibres in the cat arise in the medulla and probably from the dorsal nucleus of the vagus. The technical difficulties of demonstrating degenerative changes in very fine myelinated and unmyelinated fibres may account for the scantiness of the evidence obtained, and for the negative results recorded by some previous workers.

The blood supply of the human hypothalamus. By B. H. Dawson. *Department of Anatomy, The University, Manchester* 13

The extensive literature dealing with the anatomy, the physiology and the pathology of the human hypothalamus lacks reliable accounts of its vascular anatomy. Whilst several studies of animal material have been published, the human hypothalamic blood vessels have seldom been figured or described in detail.

An investigation of the human hypothalamic blood vessels has been undertaken as an extension of the work upon the blood vessels of the human optic chiasma reported by the writer at the Birmingham meeting of this Society last year.

The vessels were studied in microdissections, in cleared injected preparations and in nitro-prusside-benzidine preparations. Only fresh human material was employed.

The supraoptic and paraventricular nuclear regions were noted as the most richly vascularized portions of the hypothalamus and the significance of this will be discussed.

Examination of the venous patterns of the interpeduncular region indicated that in published illustrations of the hypothalamic vessels, veins are commonly depicted as arteries.

A constantly situated pre-infundibular venous arcade was noted in many specimens. Descriptions of this venous arch cannot be found in the literature.

Particular attention was devoted to the arterial and venous interrelationship between the infundibulum and the hypothalamus. It was found that, whilst the human infundibulum shares its source of arterial supply with that of the hypothalamus, no significant venous connexions exist between them.

The findings of Wislocki and of Popa and Fielding were examined in the light of these recent studies.

Observations on the departure of substances from the spinal theca. By F. Howarth. *Department of Anatomy, The University, Manchester* 13

With the aid of radio-active isotopes, the behaviour of various substances introduced into the lumbar thecae of cats has been studied. Each substance showed a rapid decline in local concentration, and a number of possible routes of departure were investigated.

It has been shown, contrary to contemporary belief, that cephalic spread within the cerebrospinal fluid with subsequent absorption by the cranial arachnoid villi, is of little significance in the reduction of local concentration of the introduced substance; yet the latter appears in the general circulation within half a minute of its intrathecal injection.

Some local system of drainage was therefore suspected, and of the various routes examined, direct venous drainage was the most striking. It was found that the azygos vein carried a higher concentration of the injected substance than any other vein examined.

Compounds of bromine, sodium and phosphorus, and also a radio-active spinal anaesthetic were employed in this study. The implications of a direct venous drainage of the lumbar theca with regard to the therapeutic administration of penicillin by this route are being examined.

The blood supply of the lungs. By E. W. WALLS. *Department of Anatomy, Middlesex Hospital Medical School, London, W.* 1

The relative vascularity of the lobes of the lungs was investigated in several ways. Direct and radiological examination of stillborn and adult lungs, cleared in wintergreen oil following the injection of a radio-opaque suspension either into the pulmonary artery (direct) or into the bronchial artery (via aorta), gave no evidence that any part of either lung was more, or less, vascular than any other part. Preparations of other lungs gave no histological evidence of variation in vascularity throughout the lung. The possibility of a functional variation in blood supply to the upper half compared with the lower half of the lung was considered, however, and investigated as follows.

In healthy male students aged about 20 years antero-posterior radiographs were taken in the inspiratory phase (1) upright, (2) lying down. Tube distance and angle of inclination were exactly the same in each exposure and the two films were developed together. As closely comparable areas of the films as possible were then examined to see whether any difference in density could be found. Density was assessed either by naked eye or by directing a beam of light through the film on to a photoelectric cell and taking readings against a galvanometer. It was found that the upper half of the lung field increased in density and the lower half decreased in density on lying down. This finding agrees with that of Laurell (1937), who described an orthostatic arterial anaemia of the upper parts of the lungs, demonstrable radiologically, which he felt was of the greatest importance with regard to the incidence of pulmonary tuberculosis.